FORM PCT/RO/101 (REV 11-98) — AMERICE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
PG3576USW	09/806892

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/03284	5 October 1999	5 October 1998

TITLE OF INVENTION
CHEMICAL CONTRUCTS AND THEIR USES

APPLICANT(S) FOR DO/EO/US
McKEOWN, Stephen, Carl
WATSON, Stephen, Paul

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☐ Other items or information:

PCT/RO/101 Request

Express Mail
EL395942305US

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/806892	PCT/GB99/03284	PG3576USW

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1,000.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	**$860.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$710.00**
☒	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$690.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$690.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	37 - 20 =	17	x $18.00	**$306.00**	
Independent claims	1 - 3 =	0	x $80.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,126.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	**$0.00**	
SUBTOTAL =				**$1,126.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**	
TOTAL NATIONAL FEE =				**$1,126.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
TOTAL FEES ENCLOSED =				**$1,126.00**	
				Amount to be: refunded	$
				charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **07-1392** in the amount of **$1,126.00** to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **07-1392** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

23347
PATENT TRADEMARK OFFICE

SIGNATURE

Frank P. Grassler
NAME

31,164
REGISTRATION NUMBER

4/5/2001
DATE

09/806892
JC02 Rec'd PCT/PTO 05 APR 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Aplication of:	MCKEOWN et al.
International Application No.:	PCT/GB99/03284
International Filing Date:	05 October 1999
Title:	CHEMICAL CONSTRUCTS AND THEIR USES

Commissioner for Patents
Washington, DC 20231

FIRST PRELIMINARY AMENDMENT

Dear Sir:

The above-identified application is being transmitted herewith for entry in the US National Phase under Chapter II of the PCT for the purpose of adding the priority information. Please amend the application as follows:

In the Abstract

Please substitute the attached Abstract, which has been placed on a separate sheet of paper according to US practice, as required under 37 CFR 1.72(b).

In the Specification

On the first line of the specification, after the Title, please add:

--This application is filed pursuant to 35 U.S.C. §371 as a United States National Phase application of International Application No. PCT/GB99/03284 filed 5 October 1999, which claims priority from GB9821669.0 filed 5 October 1998.--

In the Claims:

Please amend claim 6, 12, 13, 14, 17, 20, 21, 22, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34 and 36 as follows:

6. (Amended) A chemical construct according to claim 1 wherein each solid support contains a coding tag or coding sequence which encodes information indicative of at least part of the synthesis history of the construct.

12. (Amended) A chemical construct according to claim 1 wherein a proportion of the total substrate R in the construct is linked to the solid support by means of a connecting group Y^b having a cleavage site which is cleavable to release a fragment F^b from the solid support, the fragment F^b comprising the substrate R and at least a portion of the connecting group

Y^b; the connecting group Y^b not being cleavable to release substrate R under conditions effective to cleave the second cleavage sites in the groups Y^1R and Y^2R and wherein:
(i) the chemical fragment F^b contains a sensitising group G which sensitises the chemical fragment F^b to instrumental, e.g. mass spectroscopic analysis and/or:
(ii) the fragment F^b contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

13. (Amended) A chemical construct according to claim 1 wherein the sensitising group G is generated by cleavage at the first cleavage site of the group Y^1 or Y^2 or, when present, Y^a or the said cleavage site of Y^b.

14. (Amended) A chemical construct according to claim 1 wherein the sensitising group G is a basic amino group or a carboxylate group, preferably a basic amino group.

17. (Amended) A construct according to claim 1 wherein the fragment Fr and (where present) optionally the fragment F^a and (where present) optionally the fragment F^a contain a means for imparting a characteristic signature to the mass spectrum of the fragment.

20. (Amended) A chemical construct according to claim 18 wherein the isotopic label comprises an atom or atoms selected from $^1H/H^2$ (D), $^{79}Br/^{81}Br$, $^{12}C/^{13}C$, $^{14}N/^{15}N$ and $^{16}O/^{18}O$.

21. (Amended) A chemical construct according to claim 18 wherein the isotopic label(s) is/are located between the first and second cleavage sites of the groups Y^1 and Y^2.

22. (Amended) A chemical construct as defined in claim 1 wherein the first and second cleavage sites in the groups Y^1 are defined by first and second linker groups L^1 and L^2, first and second cleavage sites in the group Y^2 are defined by first and second linker groups L^1 and L^3, the cleavage site in the group Y^a (where present) is defined by a linker group L^a and the cleavage site in the group Y^b (where present) is defined by a linker group L^b.

24. (Amended) A chemical construct according to claim 22 wherein a spacer group A is interposed between each pair of first and second linker groups, or between the linker group L^a and the coding tag, or between the linker group L^b and the substrate R, the spacer group A containing an isotopic peak splitting label.

25. (Amended)A chemical construct according to claim 1 having a formula selected from the group consisting of:

$$\begin{array}{l} \quad L^1\text{-}A\text{-}L^2\text{-}R \\ Q\text{-}L^1\text{-}A\text{-}Tag \\ \quad L^1\text{-}A\text{-}L^3\text{-}R \end{array}$$

$$\begin{array}{l} \qquad\qquad\qquad\quad L^1\text{-}A\text{-}L^2\text{-}R \\ R\text{-}A\text{-}L^1\text{-}Q\text{-}L^1\text{-}A\text{-}Tag \\ \qquad\qquad\qquad\quad L^1\text{-}A\text{-}L^3\text{-}R \end{array}$$

$$\begin{array}{l} \quad L^1\text{-}A\text{-}L^2\text{-}R \\ Q\text{-}L^1\text{-}A\text{-}R \\ \quad L^1\text{-}A\text{-}L^3\text{-}R \end{array}$$

$$\begin{array}{l} \qquad\qquad\qquad\quad L^1\text{-}A\text{-}L^2\text{-}R \\ R\text{-}A\text{-}L^1\text{-}Q \\ \qquad\qquad\qquad\quad L^1\text{-}A\text{-}Tag \end{array}$$

$$Tag\text{-}A\text{-}L^1\text{-}Q\text{-}L^1\text{-}A\text{-}L^2\text{-}R$$

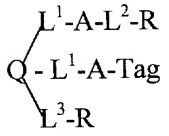
$$R\text{-}A\text{-}L^1\text{-}Q\text{-}L^1\text{-}A\text{-}L^2\text{-}R$$

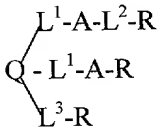
$$\begin{array}{l} \quad L^1\text{-}A\text{-}L^2\text{-}R \\ Q\text{-}A\text{-}R \\ \quad L^1\text{-}A\text{-}L^3\text{-}R \end{array}$$

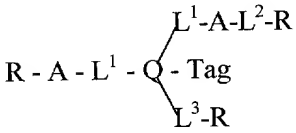
$$\begin{array}{l} \qquad\qquad\qquad\quad L^1\text{-}A\text{-}L^2\text{-}R \\ R\text{-}A\text{-}L^1\text{-}Q\text{-}A\text{-}Tag \\ \qquad\qquad\qquad\quad L^1\text{-}A\text{-}L^3\text{-}R \end{array}$$

$$\begin{array}{l} \qquad\qquad\qquad\quad L^1\text{-}A\text{-}L^2\text{-}R \\ R\text{-}A\text{-}L^1\text{-}Q \\ \qquad\qquad\qquad\quad A\text{-}Tag \end{array}$$

$$\begin{array}{l} \qquad\qquad\qquad\quad L^1\text{-}A\text{-}L^2\text{-}R \\ R\text{-}A\text{-}L^1\text{-}Q\text{-}L^1\text{-}A\text{-}Tag \\ \qquad\qquad\qquad\quad L^3\text{-}R \end{array}$$







wherein L^1, L^2, L^3, A and R are as defined in claim 1 and "Tag" represents a coding sequence.

26. (Amended) A construct as claimed in claim 1 for use in a tiered release method of screening, the construct having the formula Tag - A - L^1 - Q - L^1 - A -L^2 - R wherein Tag, A, L^1, Q ,L^2 and R are as defined claim 1.

27. (Amended) A chemical construct according to claim 1 wherein the orthogonally cleavable cleavage sites can be cleaved by a reactions selected from acid catalysed cleavage, base catalysed cleavage, oxidative cleavage, reductive cleavage, nucleophilic displacement, electrophilic displacement, and thermal, photochemical and enzymatic cleavage.

28. (Amended) Intermediate chemical constructs for use preparing a chemical construct as defined in claim 1, the intermediate constructs having the formulae $Y^{1\prime}$-Q-$Y^{2\prime}$, RY^1-Q-$Y^{2\prime}$and $Y^{1\prime}$-Q-Y^2R wherein $Y^{1\prime}$ and $Y^{2\prime}$ are reactive or protected forms of the group Y; and R, Q and Y are as defined in claim 1.

29. (Amended) Intermediate constructs of the formulae $L^{2\prime}$-A-L^1-Q-L^1-A^p, R-L^2-A-L^1-Q-L^1-A^p, $L^{3\prime}$-A-L^1-Q-L^1-A^p, R-L^3-A-L^1-Q-L^1-A^p, R-L^3-A-L^1-Q-L^1-A-$L^{2\prime}$ and $L^{3\prime}$-A-L^1-Q-L^1-A-L^2-R wherein $L^{1\prime}$, $L^{2\prime}$ and $L^{3\prime}$ are reactive or protected forms of the linker groups L^1, L^2 and L^3, A^p is a reactive or protected form of the spacer group A containing a peak splitting isotopic label, and Q, R, A, L^1, L^2 and L^3 are as defined in claim 1.

31. (Amended) An intermediate construct according to claim 29 wherein the solid support has bonded thereto a coding tag sequence L^1-A-Tag and/or a sequence R - A - L^1 -, or a precursor form thereof..

32. (Amended) A differential release method of assaying a chemical library for biological activity, the method comprising:

(i) subjecting a construct comprising a solid support Q having linked thereto groups Y^1R and Y^2R as defined in claim 1 to cleavage conditions effective to release substrate R from the group Y^1R;

(ii) testing the substrate R released from the group Y^1R in a biological assay;

(iii) subsequently subjecting the construct to cleavage conditions effective to release substrate R from the group Y^2R; and

(iv)) testing the substrate R released from the group Y^2R in a biological assay.

33. (Amended) A tiered release method of assaying a chemical library for biological activity, the method comprising:

(i) subjecting a construct as claimed in claim 1 to cleavage conditions effective to release a first portion of the substrate R from the group Y^1R;

(ii) testing the first portion of substrate R released from the group Y^1R in a biological assay;

(iii) subjecting the construct to cleavage conditions effective to release a second portion of the substrate R from the group Y^1R; and

(iv) testing the second portion of substrate R released from the group Y^1R in a biological assay.

34. A method of determining the identity of a substrate R linked to a solid support Q of a construct as claimed in claim 8 by mass spectrometric means; the solid support Q having a coding sequence attached thereto by means of a connecting group Y^a having a cleavage site cleavable to release a fragment F^a from the solid support, the fragment F^a comprising the coding sequence and at least a portion of the connecting group Y^a, wherein (i) the chemical fragment F^a contains a sensitising group G which sensitises the chemical fragment F^a to mass spectroscopic analysis;

the coding sequence comprising a sequence of coding groups the nature and order of which is indicative of the identity of the substrate R;

the method comprising cleaving the connecting group Y^a so as to release the fragment F^a from the solid support; subjecting the fragment Y^a to mass spectrometry under conditions effective to bring about mass spectral fragmentation of the coding group and the formation of mass spectral fragment ions corresponding to the loss of one

or more coding groups from the coding sequence, and thereafter correlating mass spectral peaks of the mass spectral fragment ions with the molecular ion of the fragment Y^a to identify the sequence of the individual coding groups.

36. (Amended) A method of A method of identifying a pharmaceutically useful substrate comprising preparing a library containing a plurality of chemical constructs as defined in claim 1, and subjecting the library to biological testing to identify biologically active substrates.

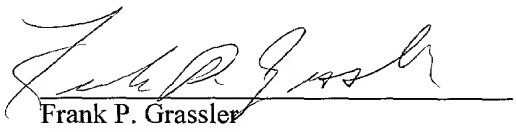
REMARKS

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Applicants have attached an abstract on a separate sheet of paper as required by US practice. Applicants have amended the specification for purposes of adding the priority information. The claims have been amended to place them in form appropriate to US practice.

Respectfully Submitted,

Date: 4/5/2001



Frank P. Grassler
Registration No. 31,164

GlaxoSmithKline
Five Moore Drive
PO Box 13398
Research Triangle Park, NC 27709
Phone: 919-483-2482
Fax: 919-483-7988

CHEMICAL CONSTRUCTS AND THEIR USES

Field of the Invention

This invention relates to chemical constructs for use in solid phase synthesis, and to methods of detecting and/or characterising the products of solid phase synthesis using the constructs.

Background of the Invention

Solid phase synthesis has been known for many years in the field of peptide synthesis and more recently has also been used increasingly for the synthesis of non-peptides.

Solid phase synthesis has found particular application in the field of combinatorial chemistry and the preparation of chemical libraries as potential sources of new leads for drug discovery, see for example Anthony W. Czarnik, *Analytical Chemistry News and Features*, June 1, 1998, pp 378A-386A, and *The Combinatorial Index*, Barry A. Bunin, Academic Press, San Diego 1998. A feature of combinatorial chemistry methods is that they enable very large numbers of different compounds to be prepared from a relatively limited number of molecular building blocks in a relatively small number of reactions. Combinatorial chemistry makes use of the "split and pool" approach in which a suspension of chemical starting material tethered to a solid support is split into N portions, each of which is reacted with a different reagent. The products of the N reactions are then pooled and mixed thoroughly, the resulting pool is split into N' portions and again each portion is reacted with a different reagent. This procedure can be repeated as many times as there are steps in the reaction sequence. Thus, for a three step reaction sequence, if the reaction mixture is divided into ten portions at each stage, each of the pools being reacted with a different reagent before recombining with the other portions, the total number of compounds formed by the process will be $10^3 = 1000$. Thus it can be seen that by using the split and pool technique, a large number of different molecules can be synthesised using a minimal number of reactions (thirty in the case mentioned above).

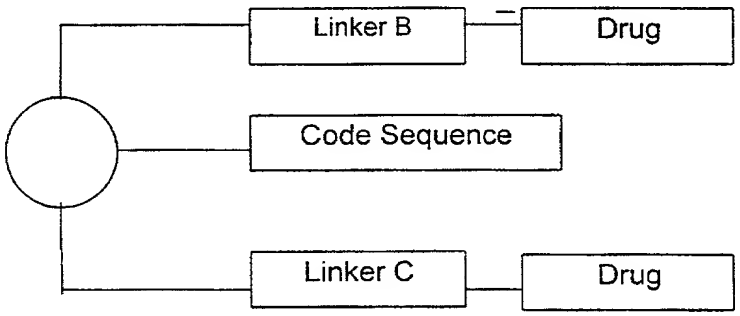
Each of the solid supports (e.g. a resin bead) will have a single product molecule

tethered to it and hence in principle each of the reaction products can be separated and analysed or subjected to biological testing simply by isolating each single solid support and cleaving the product from the support. However, the numbers of compounds generated in large libraries by combinatorial methods means that it can be impracticable to identify and characterise each compound. Consequently, the compounds usually are first tested, either on the solid support or after cleaving from the support, and only those compounds which show some biological activity are subsequently identified. In order to minimise the number of biological tests carried out, compounds can be tested in pools containing a predetermined number of compounds, the inactive pools being discarded and the active pools being subject to further investigation. The biological activities of the compounds can be analysed using high throughput automated assay techniques permitting large numbers of compounds to be analysed in a short time.

One of the problems facing the chemist is how to identify and characterise the various compounds in a combinatorial library since each compound will be present in the library only at very low concentrations, and there will usually be insufficient compound present on a given solid support to allow for both biological testing and identification of the compound. This problem has been addressed by providing each solid support with a coding tag from which the identity of the compound can be determined. Thus, for example, a coding tag can be built up in sequential steps on the solid support in parallel with the construction of the desired target compound, the coding tag reflecting the synthesis history of the product compound and being unique for each product compound. The coding tag is usually built up on the support using chemical reactions of a type which are orthogonal to the chemistry used to build up the product compound, thereby ensuring that the coding units and product compounds do not become confused. Once the product compound has been tested, and its biological activity confirmed, the coding tag can then be decoded to allow identification of the compound.

Where a product compound has been subjected to a preliminary biological screen as part of a pool of compounds, and the pool has been shown to have activity, it is then necessary either to look at smaller pools of compounds or individual compounds in order to discover the identity of the active compound. In many assay systems, it is necessary to cleave the compound from the solid support in order to carry out biological testing, and this can lead to problems with the pool testing approach. Thus, if all of the active compound has been cleaved from the solid support in order to carry out the testing, such that the pool contains a mixture of a large number of dissolved compounds, recovery of each individual compound

for further testing would create a severe separation problem. In order to overcome or avoid this problem, "differential release" constructs have been created in which the product compound, e.g. a drug molecule, is linked to the solid support by several different types of linker groups, each of the linker groups being orthogonally and selectively cleavable under different chemical conditions. Such a construct (Construct A) is shown schematically below.



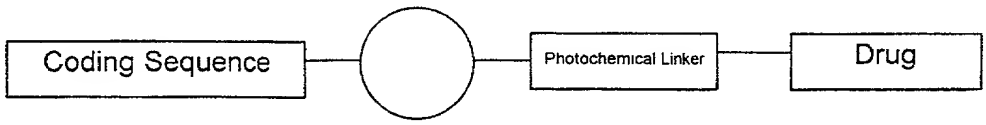
Construct A

Following a combinatorial synthesis scheme, the reaction products can be divided into x pools each containing y solid supports, each solid support bearing a different product drug molecule. Each pool can then be subjected to cleavage conditions suitable for selectively cleaving linker B so as to free a proportion of the total drug present on the solid support. The solid supports can then be removed and the residual solution subjected to the desired biological assays. If the pool as a whole shows biological activity, the individual partially cleaved solid supports in that active pool can then be separated into individual containers and subjected to conditions effective to bring about cleavage of the second linker, linker C. Subsequently, the individual drugs can each be screened for biological activity.

Once biological activity has been observed for drug associated with a particular individual solid support, the support is subjected to conditions which allow the tagging code to be decoded and the identity of the drug compound established.

An alternative to "differential release" is "tiered release". In a tiered release system, the construct does not employ two orthogonal cleavage sites as with the differential release constructs, but makes use of only a single cleavage site. A proportion of the drug or test compound is released in a first cleavage step to provide sufficient compound for a first biological assay, and subsequently more drug can be released by cleavage at the same cleavage site for further biological assays. Such tiered release systems make use of cleavage

reactions which are relatively slow, and where the rate of cleavage can be reasonably well controlled. Photochemical cleavage is typically used in tiered release systems. A construct for use in a tiered release system is shown below as Construct B.



Construct B

In any solid phase synthesis procedure, whether or not part of a combinatorial procedure, it is important to be able to determine the optimal conditions for a given reaction step in a series of steps. It is also important to be able to monitor the progress or path of a reaction so that it can be determined whether a particular reaction has gone to completion, or indeed whether a particular reaction has taken place at all. This is particularly important in a multistage solid phase synthesis where the failure of a given stage to proceed to completion can lead to the formation of side products thereby complicating what is otherwise a relatively straightforward separation procedure.

Although both invasive and non-invasive analytical methods are known for determining the products of solid phase synthesis (see *The Combinatorial Index, idem*), one of the recognised problems with solid phase synthesis is that it is generally more difficult to monitor the progress of a reaction; see for example the Czarnik paper referred to above. This is particularly true with the products of combinatorial chemistry methods where there is a need to use rapid high throughput techniques to analyse the large numbers of compounds generated by such methods. Techniques such as mass spectrometry are potentially ideally suited as a means of providing high throughput analyses, but a substantial problem is that not all compounds produce a guaranteed response under mass spectrometric conditions. Indeed, many compounds are "invisible" to the so-called "soft" methods of mass spectrometry such as Matrix-Assisted Laser-Desorption Ionisation (MALDI) and Electrospray mass spectrometry which are intended to detect molecular ions without causing significant fragmentation of the molecule. One of the reasons for this is that under MALDI and electrospray conditions, many compounds, particularly peptides, do not ionise sufficiently well to give a detectable mass spectral response.

It is an object of the present invention to provide a means of monitoring the progress or path of a chemical reaction on a solid support, for example in a combinatorial synthesis,

which avoids problems inherent in known methods and which provides a means of detecting and identifying the products of solid phase synthetic techniques using mass spectroscopy.

Accordingly, in a first aspect, the invention provides a chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto groups Y^1R and Y^2R; wherein R is a substrate or a coding tag and the groups Y^1 and Y^2 are connecting groups each having a first cleavage site, at least one of Y^1 and Y^2 having a second cleavage site located between the first cleavage site and group R, the first cleavage site being orthogonally and selectively cleavable with respect to the second cleavage site, and, when both groups Y^1 and Y^2 contain a second cleavage site, the second cleavage site in Y^1 being selectively and orthogonally cleavable with respect to the second cleavage site in Y^2; the second cleavage site being cleavable to release the substrate; and the first cleavage site being selectively cleavable to release a fragment Fr comprising the substrate R and at least a portion of the connecting group Y; and wherein:

(i) the chemical fragment Fr contains a sensitising group G which sensitises the chemical fragment Fr to instrumental, e.g. mass spectroscopic analysis and/or:

(ii) the fragment Fr contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

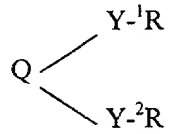
Preferably at least one group R is a substrate and more preferably the substrate is a drug molecule or other molecule having biological activity.

In one preferred embodiment, there is provided a chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto groups Y^1R and Y^2R; wherein R is a substrate (such as a drug molecule) and the groups Y^1 and Y^2 are connecting groups each having first and second cleavage sites which are orthogonally and selectively cleavable, the second cleavage site in Y^1 being selectively and orthogonally cleavable with respect to the second cleavage site in Y^2; the second cleavage site being cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr comprising the substrate R and at least a portion of the connecting group Y; and wherein:

(i) the chemical fragment Fr contains a sensitising group G which sensitises the chemical fragment Fr to instrumental, e.g. mass spectroscopic analysis and/or:

(ii) the fragment Fr contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

The constructs of the invention can be illustrated by the general formula:



The constructs of the invention provide a differential release system which is more readily analysable to determine the outcome of individual reactions on the solid support. Thus, for example, by subjecting the construct to cleavage conditions effective selectively to bring about cleavage at each of the first cleavage sites, chemical fragments containing the substrates R linked to an "analytical lever" are produced in which the detectability of the chemical fragments, and hence the substrates, is enhanced. Thus, cleavage at the first cleavage sites can be used to determine whether a particular substrate has been formed, a facility which is useful as a means of carrying out quality control (QC) during a reaction scheme or library generation, and which provides a means of analysis that can be used when optimising the conditions for a given synthetic process step.

The constructs can contain further groups Y^n, each of which has a second cleavage site which is selectively and orthogonally cleavable with respect to the second cleavage sites in the other Y groups. Thus, for example, the constructs can contain a group Y^3Q in which the second cleavage site in the group Y^3 is cleaved under different chemical conditions from the second cleavage sites in Y^1R and Y^2R. Thus by means of the invention, it is possible to provide differential release of product substrate molecules (e.g. drugs) in two, three, four or more steps, thereby assisting the products of large combinatorial libraries to be screened biologically in pools of manageable size.

In a second aspect, the invention provides a differential release method of assaying a chemical library for biological activity, the method comprising:

(i) subjecting a construct comprising a solid support Q having linked thereto groups Y^1R and Y^2R as hereinbefore defined to cleavage conditions effective to release substrate R from the group Y^1R;

(ii) testing the substrate R released from the group Y^1R in a biological assay;

(iii) subsequently subjecting the construct to cleavage conditions effective to release substrate R from the group Y^2R; and

(iv)) testing the substrate R released from the group Y^2R in a biological assay.

In addition to providing a differential release method of analysing a library, the

invention also provides a method of tiered release analysis which makes use of constructs which are selectively cleavable to release a fragment Fr comprising the substrate R and at least a portion of a connecting group Y; and wherein:

(i) the chemical fragment Fr contains a sensitising group G which sensitises the chemical fragment Fr to instrumental, e.g. mass spectroscopic analysis and/or:

(ii) the fragment Fr contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

Accordingly, in a further aspect, the invention provides a tiered release method of assaying a chemical library for biological activity, the method comprising:

(i) subjecting a construct comprising a solid support Q having linked thereto a group Y^1R as hereinbefore defined to cleavage conditions effective to release a first portion of the substrate R from the group Y^1R;

(ii) testing the first portion of substrate R released from the group Y^1R in a biological assay;

(iii) subjecting the construct to cleavage conditions effective to release a second portion of the substrate R from the group Y^1R; and

(iv) testing the second portion of substrate R released from the group Y^1R in a biological assay.

In each of the above differential release and tiered release methods of the invention, the cleavage step (i) is typically performed on a pool of solid supports, each bearing a different substrate R and, following the cleavage step (I), the solid support is typically separated from the released substrate R. If biological activity is detected in the pool, the solid supports can be isolated, or grouped into smaller pools, and then subjected to cleavage step (iii) to release further substrate for testing so that the biological activity can be traced to a particular solid support or small group of supports.

To assist in determining the identities of the products of a combinatorial synthesis scheme, each solid support can contain a coding tag or coding sequence which encodes information indicative of at least part of the synthesis history of the construct. The coding tag can be built into the solid support itself or a coding sequence can be linked to the solid support. The coding sequence preferably is linked to the solid support by means of a connecting group Y^a having a cleavage site cleavable to release a fragment F^a from the solid support, the fragment F^a comprising the coding sequence and optionally at least a portion of

the connecting group Y^a.

It is preferred that:

(i) the chemical fragment F^a contains a sensitising group G which sensitises the chemical fragment F^a to instrumental, e.g. mass spectroscopic analysis and/or:

(ii) the fragment F^a contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

The coding tag can take the form of a group or sequence of coding groups that can be fragmented, and in particularly sequentially fragmented, under mass spectrometric conditions to provide one or more characteristic mass spectral peaks that provide a code from which the substrate can be identified. For example, the coding tag can comprise a sequence of different amino acids, e.g. two, three or four amino acids, the identity and order of the amino acids forming a code indicative of the identity of the substrate. Under mass spectrometry conditions, the coding groups (e.g. amino acids) can be sequentially cleaved to give a series of characteristic mass spectral fragments, relating to coding tags in which ,for example, one or two or more amino acids have been lost from the tag. By relating the masses of the fragment peaks back to the mass of the parent coding tag, it is possible to identify the individual coding groups (e.g. amino acids) and their position on the coding tag, thereby enabling the code to be broken and the substrate identified. By providing a sensitising group G and a means for imparting a characteristic signature to the mass spectrum, the present invention provides a means firstly of ensuring that the amino acids or other coding groups are sufficiently ionised to give strong mass spectral peaks, and secondly of ensuring that each of the characteristic mass spectral fragments formed from the fragmentation of the coding sequence can be distinguished from extraneous peaks.

Accordingly, in another aspect, the invention provides a method of determining the identity of a substrate R linked to a solid support Q by mass spectrometric means; the solid support Q having a coding sequence attached thereto by means of a connecting group Y^a having a cleavage site cleavable to release a fragment F^a from the solid support, the fragment F^a comprising the coding sequence and at least a portion of the connecting group Y^a, wherein (i) the chemical fragment F^a contains a sensitising group G which sensitises the chemical fragment F^a to mass spectroscopic analysis; and preferably

(ii) the fragment F^a contains a means for imparting a characteristic signature to the mass spectrum of the fragment;

the coding sequence comprising a sequence of coding groups the nature and order of which is indicative of the identity of the substrate R;

the method comprising cleaving the connecting group Y^a so as to release the fragment F^a from the solid support; subjecting the fragment Y^a to mass spectrometry under conditions effective to bring about mass spectral fragmentation of the coding group and the formation of mass spectral fragment ions corresponding to the loss of one or more coding groups from the coding sequence, and thereafter correlating mass spectral peaks of the mass spectral fragment ions with the molecular ion of the fragment Y^a to identify the sequence of the individual coding groups

In the methods and constructs of the invention, as an alternative, or in addition, to including a coding tag which is chemically different from the substrate, a proportion of the substrate itself can be bonded to the solid support in such a way that it is not cleaved from the support under the conditions used to remove the rest of the substrate from the support. The remaining substrate can thus function as an analytical label enabling identification by a method such as mass spectrometry. More particularly, a proportion of the total substrate R in the construct can be linked to the solid support by means of a connecting group Y^b having a cleavage site which is cleavable to release a fragment F^b from the solid support, the fragment F^b comprising the substrate and at least a portion of the connecting group Y^b; the connecting group Y^b not being cleavable to release substrate R under conditions effective to cleave the second cleavage sites in the groups Y^1R, Y^2R and Y^n [if present]) and wherein:

(i) the chemical fragment F^b contains a sensitising group G which sensitises the chemical fragment F^a to instrumental, e.g. mass spectroscopic analysis and/or:

(ii) the fragment F^b contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

Thus, after liberation of the main proportion of the substrate from the constructs for biological testing, fragment F^b can be selectively cleaved from the solid support to yield sensitised substrate which is readily analysable by methods such as mass spectrometry. This could be done, for example, after biological assays had been carried out on the main proportion of the substrate, biological activity having been found, and it was required to determine the identity of the substrate. Alternatively, fragment F^b can be cleaved from the solid support without first removing the other substrate from the solid support, for example as part of a quality control (QC) procedure to test the quality of the products of the solid phase synthesis. In this latter procedure, fragment F^b can be cleaved from the solid support along

with fragments Fr and F^a , analysis of the fragments Fr, F^a and F^b providing a full picture of the outcome of the synthesis.

The cleavage site of groups Y^a and Y^b is preferably cleavable under conditions corresponding to those needed to cleave the first cleavage sites in the groups Y^1R and Y^2R. Thus, the product or substrate-containing fragments and the coding tag-containing fragment can be cleaved from the solid support at the same time thereby permitting them to be analysed (e.g. by mass spectrometry) simultaneously.

The chemical fragment F^a preferably contains a sensitising group G which sensitises the chemical fragment F^a to instrumental, e.g. mass spectroscopic analysis.

In a preferred aspect of the invention, the construct is characterised in that the sensitising group G is generated by cleavage at the first cleavage site(s) of the groups Y^1, Y^2, Y^n ,Y^a or Y^b respectively. In this preferred embodiment of the invention, a moiety containing the sensitising group G is formed or introduced at the first cleavage site(s) by cleavage of the "skeleton" of the construct, as distinct from cleavage of a side chain or mere removal of a protecting group.

The sensitising group G renders the fragment Fr, F^a or F^b, and hence the substrate R, coding tag or substrate-containing coding tag, more sensitive to analysis by a given analytical technique, and in particular a mass spectrometric technique. Thus the sensitising group can be a group which is readily ionisable under the conditions encountered in a mass spectrometer, and in particular electrospray mass spectrometry, to afford a strong signal.

The ionisable sensitising group serves to ensure that the fragment Fr, F^a or F^b is ionised sufficiently in the mass spectrometer to give a strong response. This overcomes a problem inherent in many molecules synthesised by solid phase methods where a suitable ionising group is not present and analysis by high throughput mass spectral techniques is problematical.

The ionisable group can be for example a basic amino group or a carboxylate group but preferably it is a basic amino group. It will be appreciated that the term "basic amino group" as used herein refers in particular to an amino group which is readily protonated.

The basic amino group can be a primary amino group, a secondary amino group, or a tertiary amino group. Where the basic amino group is a tertiary amino group, it can be for example, a cyclic amino group such as piperidino, piperazino, pyrrolidino, or morpholino, piperidino or piperazino (e.g.N-methylpiperazino) being presently preferred.

In accordance with a preferred embodiment of the invention, a moiety containing the sensitising group G, such as a basic amino group, is formed or introduced at the cleavage site as the chemical fragment Fr, fragment F^a or F^b (hereinafter collectively referred to as the analytical fragment) is cleaved from the solid support. The advantage of forming the sensitising group G in this way is that it avoids the potential problem of a pre-existing or preformed sensitising group interfering with the chemistry of the construct.

The atoms or functional group making up the sensitising group G can be present in a masked form in the construct before cleavage of the analytical fragment from the resin, the cleavage conditions merely serving to unmask the sensitising group G. Alternatively, the atoms or functional group making up or containing the sensitising group G can be introduced at the cleavage site during the cleavage reaction. For example, where the sensitising group is a basic amino group, the nitrogen atom of the amino group can be present in the construct before cleavage, or it can be introduced during the cleavage reaction.

When the sensitising group G is introduced from an external source during the cleavage reaction, it can form part of a larger moiety. For example, the group G can be introduced as part of a group X-G wherein X is the residue of a nucleophile or electrophile, for example a nitrogen-based or sulphur-based nucleophile. Examples of groups X-G include groups of the formula G-Alk-Nuc wherein Alk is an alkylene group and Nuc is a nucleophile such as a secondary amino group (e.g. the secondary amino group in N-methylpiperazine) or a thiolate group.

It is preferred that the analytical fragment contains a means for imparting a characteristic signature to the mass spectrum of the fragment. The signature can advantageously be provided by incorporating into the fragment a "peak splitting" isotopic label. The peak splitting isotopic label comprises at least one atom that exists in a number of stable isotopic forms. By isotopic labelling of one or more particular atoms in the analytical fragment such that a given atom is labelled with a mixture of isotopes, the mass spectra of the molecular ions will appear as a characteristic pattern, the precise pattern depending on the

relative amounts of the individual isotopes. Thus, for example, if a given atom in the fragment Fr is labelled such that 50% of the atoms are of one isotopic form and 50% are of another isotopic form, the mass spectrum will show the molecular ion as a characteristic doublet in which the peaks are of approximately equal height.

The purpose of the peak splitting atom(s) is to provide a characteristic pattern which will characterise any peak in the mass spectrum originating from the analytical fragment Fr and/or F^a, and/or F^b thereby distinguishing such peaks from those due to extraneous materials.

The analytical fragments Fr, F^a and F^b can be labelled the same or differently but in one preferred embodiment, they are labelled differently so as to produce different characteristic signatures. In this way, mass spectral peaks due to the coding tag can be distinguished from mass spectral peaks from the fragments containing the substrate.

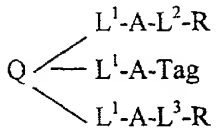
Examples of atoms that can be used as isotopic peak splitting labels include $^1H/H^2$ (D), $^{79}Br/^{81}Br$, $^{12}C/^{13}C$, $^{14}N/^{15}N$ and $^{16}O/^{18}O$.

The analytical fragments Fr, F^b and F^a can each contain a single peak splitting isotopic label or more than one such label. For example, the isotopic label can be a single bromine atom in which case the peak for the molecular ion of the analytical fragment liberated following cleavage from the solid support will appear as a doublet. By introducing a second or subsequent peak splitting label(s), a more complex peak pattern will be produced for the molecular ion.

The isotopic peak splitting label(s) preferably is/are located between the first and second cleavage sites of the groups Y^1 and Y^2.

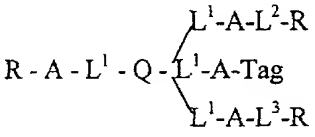
The first and second cleavage sites in the groups Y^1 can be defined by first and second linker groups L^1 and L^2, the first and second cleavage sites in the group Y^2 can be defined by first and second linker groups L^1 and L^3, and the cleavage site in the group Y^a can be defined by a linker group L^a, which preferably corresponds to L^1, L^1 ,L^2 and L^3 being selectively and orthogonally cleavable. A "spacer group" A can be interposed between each pair of first and second linker groups, or between the linker group L^a and the coding tag, the spacer group A typically containing an isotopic peak splitting label.

Accordingly, one preferred embodiment of the invention can be represented by the formula:

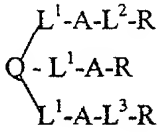


wherein "Tag" represents a coding sequence.

In another preferred embodiment, the construct has a substrate-containing analytical group R-A linked to the solid support by a linker L^1 which is orthogonally cleavable with respect to the linkers L^2 and L^3. A construct of this type can be represented by the formula:

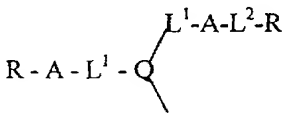


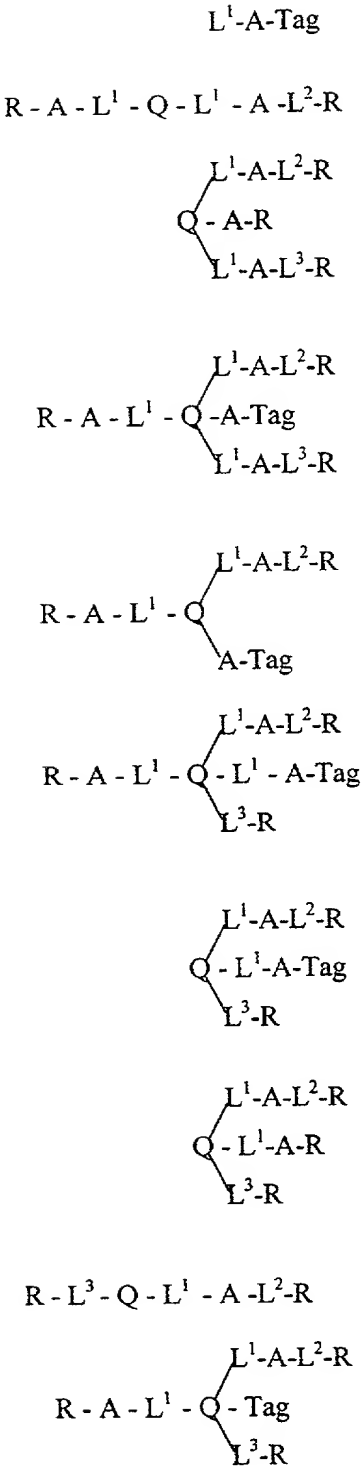
In a further preferred embodiment, the coding sequence is omitted and the means of identifying the substrate is provided by the group R - A - L^1 which can be cleaved to provide sensitised substrate for analysis by mass spectrometry. Such a construct has the formula:



In another preferred embodiment, the invention provides a construct for use in a tiered release method of screening as described above, the construct containing a sensitised coding tag. Such a construct can be represented by the formula Tag - A - L^1 - Q - L^1 - A -L^2 - R.

In other embodiments of the invention, the constructs can have a formula selected from the group consisting of:





wherein L^1, L^2, L^3, A and R are as hereinbefore defined and "Tag" represents a coding sequence.

The linkers L^1, L^2, and L^3 are orthogonally and selectively cleavable; i.e. the

conditions used to effect cleavage at the cleavage site in one linker will not cleave the other. A wide variety of different types of cleavage reaction can be used, examples being reactions selected from acid catalysed cleavage, base catalysed cleavage, oxidative cleavage, reductive cleavage, nucleophilic displacement, electrophilic displacement, and thermal, photochemical and enzymatic cleavage.

Thus, in a preferred embodiment, there is provided a chemical construct as hereinbefore defined wherein the first cleavage site (e.g. as defined by the linker L^1) is selectively cleavable by one type of chemistry selected from a group of chemistries consisting of cleavage under acid conditions, base catalysed cleavage, oxidative cleavage, reductive cleavage, nucleophilic displacement, electrophilic displacement, and thermal, photochemical and enzymatic cleavage, one of the second cleavage sites (e.g. as defined by the linker L^2) is selectively cleavable by a second and different type of chemistry selected from the said group, and the other of the second cleavage sites (e.g. as defined by the linker L^3) is selectively cleavable by a third and different type of chemistry selected from the said group.

Any one or more of the cleavage sites/linkers, can be of the "safety catch" variety; i.e. the cleavage site or linker group must be chemically modified in a first step before it can be subjected to cleavage in a second step. An advantage of such an arrangement is that it prevents or significantly reduces the possibility of cleavage taking place inadvertently. One example of a "safety catch" mechanism involves oxidation of a functional group in a first step, the oxidation serving to make the functional group more amenable to displacement by a nucleophile in a subsequent cleavage step. The nucleophile can vary considerably in structure. For example, in one embodiment, the nucleophile can be an amino group-containing nucleophile), the amino group participating in the nucleophilic displacement action such that the amino group is attached directly to the cleavage site. Alternatively, in another embodiment, the amino group (or other sensitising group) can be present in a group (e.g. a dialkylaminoalkyl-thiolate anion) containing another nucleophile such as a sulphur nucleophile which becomes attached to the cleavage site. In a further embodiment, the nucleophile can be a moiety containing a one basic nitrogen atom which functions as a nucleophile, and another basic nitrogen which functions as the sensitiser group G, an example of such a nucleophile being N-methylpiperazino.

Examples of linkers that can be selectively cleaved under acidic conditions include appropriately substituted benzyloxycarbonyl groups and substituted diphenyl-methylamino

groups, both of which can be cleaved by the action of trifluoroacetic acid.

Particular examples of acid cleavable linker groups are set forth in *The Combinatorial Index*, Barry A. Bunin, Academic Press, San Diego 1998, the disclosure of which is incorporated herein by reference. Linkers of the "Rink" or "Knorr" type typically comprise an N-protected 1-amino-1,1-diphenylmethane moiety, the amino group when deprotected allowing attachment to a substrate, one of the phenyl rings being substituted for example with dimethoxy groups and the other having a carboxyalkyloxy substituent providing a second point of attachment. Cleavage with trifluoroacetic acid gives rise to a substrate compound having a terminal carboxamido group. Linkers of the "Wang" type typically contain a substituted phenoxyacetyl group, the acetyl group providing one point of attachment, and a benzylic hydroxyl group on the phenyl ring forming a second point of attachment. Esters can be formed between a carboxyl group of a substrate and the benzylic hydroxyl group, the ester groups being subsequently cleavable with trifluoroacetic acid (TFA) to release a substrate compound having a terminal carboxylate group.

Examples of groups that can be selectively cleaved under photochemical conditions include carbamate groups such as *o*-nitrobenzyloxycarbamate groups in which initial cleavage takes place between the benzyloxy and carbonyl groups, elimination of carbon dioxide from the resulting fragment giving a basic amino group.

Examples of groups that can be cleaved by nucleophilic displacement include mercaptopyrimidine-based "safety catch" linkers such as 5-carboxy-2-mercaptopyrimidine, where cleavage can be effected by reacting under oxidising conditions to generate a sulphoxide or sulphone linkage, followed by reaction with a nucleophilic amino group to form a 2-aminopyrimidine. Particular examples of nucleophilic groups that can be used to effect cleavage of oxidised mercaptopyrimidine-based linkers include cyclic amines such as piperidine and N-substituted piperazine (e.g. N-methylpiperazine), and amino group-containing thiolate nucleophiles (e.g. dimethylaminoethylthiolate). Examples of oxidising conditions are those generated oxidising agents such as per-acids, e.g. a perbenzoic acid such as *m*-chloroperbenzoic acid, and certain inorganic per-salts such as potassium monoperoxysulphate.

By making the first and second cleavage sites, e.g. as defined by the first and second linker groups L^1 and L^2, or L^1 and ^{3} orthogonally cleavable, it is possible selectively to

separate from the construct either the analytical fragment or the substrate R, simply by using different cleavage conditions. This means that during experiments designed to optimise the conditions for a particular reaction step, the chemist can subject the construct to conditions suitable for cleaving off the analytical fragment thereby allowing analysis to be carried out to determine the outcome of each test reaction. Similarly, during a preparative scale reaction (e.g. a scale-up reaction or commercial production), quality control (QC) can be carried out by removing a number of solid supports from the reaction vessel, cleaving the constructs at the first cleavage site and analysing the resulting fragment to see whether a particular reaction step has worked. On the other hand, by cleaving at the second cleavage site e.g. on the second linker group, rather than the first, the reaction product R is released from the solid support. Thus, an advantage of the constructs of the present invention is that they can be used both at an experimental level to optimise a particular process step, and also at a preparative level without modifying the linker groups.

In one embodiment of the invention, the fragments Fr, F^a or F^b, and more preferably the spacer group A contains an alkylene diamine group or aminoalkoxy group. The precise size of the alkylene group and its degree of substitution is not currently considered to be important, but particular examples are ethylene or propylene diamine or aminoalcohol groups which may be substituted or unsubstituted. The alkylene diamine group or aminoalcohol typically contains a peak splitting isotopic label as hereinbefore defined. The two amino groups can each be bonded to respectively the first and second linker groups. In order to increase the mass of the spacer group A, or change its properties, the alkylene diamine group can be substituted by an aryl group, or example an N-aryl group such as an N-benzyl group, or the alkylene chain can be substituted (for example with one or more fluorine atoms to alter the volatility of the analytical fragment). The N-aryl group may optionally be substituted with one or more substituent groups.

Where, as is preferred, the spacer group contains one or more mass-spectral peak splitting isotopic labels, these can be located either in the alkylene chain or in a substituent group attached to the alkylene chain. Thus, for example, an N-benzyl group bonded to one of the two amino groups in an alkylenediamine can have a methylene group which is substituted with the peak splitting atom deuterium. Alternatively, an aryl ring (e.g. an N-benzyl group) present in a substituent on the alkylene diamine can be substituted with a peak splitting bromine atom, one particular example of an aryl group being an N-*o*-bromobenzyl group.

The fragments Fr, F^a or F^b, and in particular the spacer group A, as well as providing a means of identification using mass spectrometry, can also be provided with one or more additional sensitisers to allow characterisation by other analytical techniques. For example, the spacer group can contain a chromophore to allow characterisation by ultra violet (u.v.) or fluorescence spectroscopy.

Although alkylene diamine and amino alcohol groups are given as specific examples of spacer groups, alternative spacer groups can be used. For example, the spacer groups can be formed from hydrocarbon chains containing up to thirty or more carbon atoms in the chain which can be optionally interrupted with one or more heteroatoms such as oxygen, nitrogen or sulphur. As a further alternative, the spacer can be for example a peptide chain containing one or more amino acids. The precise nature and length of the spacer is not currently considered to be important provided that the spacer does not interfere with the chemistry of the construct.

The solid support Q can be any type of solid support suitable for use in solid phase synthesis, and in particular combinatorial chemistry. Thus, purely by way of example, the solid support can take the form of beads, a solid surface, solid substrates, particles, pellets, discs, capillaries, hollow fibres, needles, solid fibres, or organic or inorganic gels such as silica gels, and insoluble organic particles such as particles formed from fullerenes.

Examples of beads are polymeric beads such as cellulose beads or resin beads, particular examples of materials from which resin beads can be prepared including functionalised polymer resins such as polystyrene resins, polyacrylamide resins and dimethylacrylamide resins. Examples of suitable supports are listed in *The Combinatorial Index* by Barry A. Bunin, referred to above.

In another aspect, the invention provides a method of analysing the constructs hereinbefore defined; the method comprising cleaving the groups Y^1 and Y^2, and optionally the group Y^a to release the chemical fragment Fr (and optionally the fragments F^a and F^b and then subjecting the chemical fragments Fr, and F^a to mass spectrometry, e.g. electrospray mass spectrometry.

The analysis of the fragment Fr provides information on the reaction history of the construct. Thus, by mass spectrometric analysis, it can readily be determined whether or not

the desired substrate has been formed in a given reaction sequence. Analysis of the fragment Fr can therefore be used not only to characterise the substrate or product of the solid phase reaction sequence, but also to follow the progress of the reactions.

In a further aspect, the invention provides intermediate chemical constructs for use preparing a chemical construct as hereinbefore defined, the intermediate constructs having the formulae $Y^{1\prime}$-Q-$Y^{2\prime}$, RY^1-Q-$Y^{2\prime}$and $Y^{1\prime}$-Q-Y^2R wherein $Y^{1\prime}$ and $Y^{2\prime}$ are reactive or protected form of the group Y; and R, Q and Y are as hereinbefore defined.

In a still further aspect, the invention provides intermediate constructs of the formulae $L^{2\prime}$-A-L^1-Q-L^1-A^P, R-L^2-A-L^1-Q-L^1-A^P, $L^{3\prime}$-A-L^1-Q-L^1-A^P, R-L^3-A-L^1-Q-L^1-A^P, R-L^3-A-L^1-Q-L^1-A-$L^{2\prime}$, $L^{3\prime}$-A-L^1-Q-L^1-A-L^2-R , wherein $L^{1\prime}$, $L^{2\prime}$ and $L^{3\prime}$ are reactive or protected forms of the linker groups L^1, L^2 and L^3'L hereinbefore defined, A^P is a reactive or protected form of the spacer group A containing a peak splitting isotopic label, and Q, R, A, L^1, L^2 and L^3 are as hereinbefore defined. In a particular embodiment, the group A^P has the formula NH-Alk-NX^1 wherein Alk is an alkylene group and X^1 is hydrogen or an aralkyl group. The intermediate construct is preferably isotopically labelled with a peak splitting combination of atoms such as $^1H/H^2$ (D), $^{79}Br/^{81}Br$, $^{12}C/^{13}C$, $^{14}N/^{15}N$ and $^{16}O/^{18}O$.

In each of the aforementioned intermediates, the solid support can have optionally bonded thereto a coding tag sequence L^1-A-Tag and/or a substrate-based tag sequence R - A - L^1 -, and precursor forms thereof such as L^1-A^P .

The invention will now be illustrated but not limited by reference to the following examples.

Brief Description of the Figures

Figure 1 illustrates the mass spectrum of the product of the cleavage, by oxidation and nucleophilic displacement, of a construct of the formula Q-L^1-A-L^2-R wherein R is a model compound benzamide, L^1 is a thiopyrimidine linker group, A is an N-bromobenzyl substituted ethylene diamine "peak splitting group" and L^2 is a linker group which is cleavable under photochemical conditions.

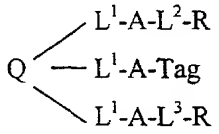
Figure 2 illustrates the mass spectrum of the construct of Figure 1 following

photochemical cleavage of the linker L^2 and subsequent oxidation / nucleophilic displacement at linker L^1.

Figure 3 illustrates the mass spectrum of the product of the cleavage, by oxidation and nucleophilic displacement, of a construct of the formula Q-L^1-A-Tag wherein L^1 is a thiopyrimidine linker group, A is the same peak splitting group as is present in the construct of Figure 1, and Tag is a coding sequence consisting of a tripeptide.

Figure 4 illustrates the mass spectrum of the cleavage product of Figure 3 but wherein the conditions within the mass spectrometer have been adjusted to cause fragmentation of the molecular ion at 716 a.m.u. in order to produce peaks due to constructs in which one or more amino acids have been lost from the molecular ion.

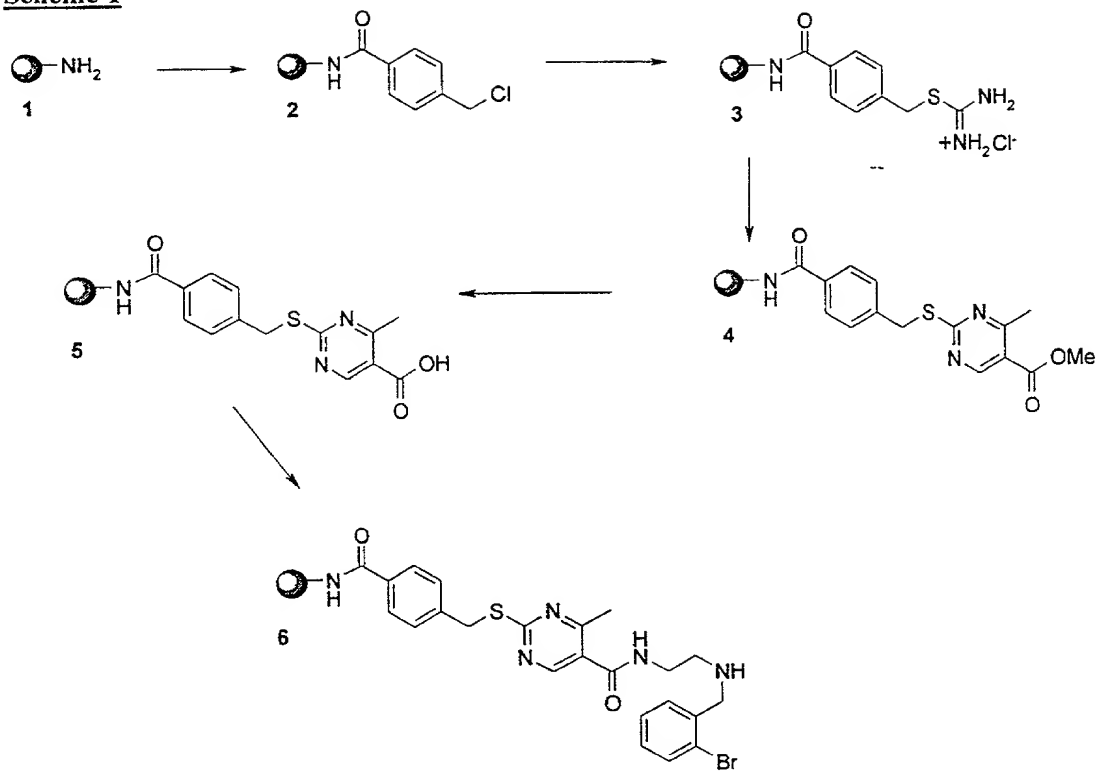
Figure 5 illustrates the mass spectrum of the product of the cleavage, by oxidation and nucleophilic displacement, of a construct of the formula:



wherein "Tag" represents an allyloxyglycine code group, L^1 , L^2 and A are the same as for the constructs of Figures 1 to 4 and L^3 is a linker of the "Rink" or "Knorr" type which is cleavable by trifluoroacetic acid.

EXAMPLE 1

Scheme 1



Experimental for Scheme 1

A solution of 4-(chloromethyl)benzoic acid (0.27 g, 1.6 mmol), Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) ® (0.83 g, 1.6 mmol) and diisopropylethylamine (0.56 ml, 3.2 mmol) in dimethylformamide (8 ml) was shaken for 20 minutes and was then added to TentaGel NH2 (Rapp Polymere) resin **1** (1 g, 0.32 mmol) . The resulting mixture was shaken for 5 hours, following which the resin was drained and washed with dimethylformamide, dichloromethane, diethyl ether, dichloromethane and finally diethyl ether . The resin (**2**) was then dried *in vacuo*.

A slurry of thiourea (0.58 g, 7.7 mmol) in dioxane (4 ml) and ethanol (1 ml) was added to Resin **2** (1 g, 0.32 mmol) and the whole heated at 80 °C for 16 hours. The resin was drained and washed with dimethylformamide (x 5), dichloromethane (x 5), diethyl ether, dichloromethane and finally diethyl ether to give resin **3**. The resin was then dried *in vacuo*.

A solution of methyl-2-(dimethylaminomethylene)-3-oxobutanoate (0.17 g, 0.96

mmol) and triethylamine (65 ml, 0.48 mmol) in dimethylformamide (10 ml) was added to resin **3** (1 g, 0.31 mmol) and the mixture was heated at 80 °C for 16 hours. The resin was then drained and washed with dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether. The washed resin (**4**) was dried *in vacuo*.

Resin **4** (0.5 g, 0.15 mmol) was then treated with a 1:1 mixture of tetrahydrofuran and 2N aqueous sodium hydroxide (4 ml) and shaken for 2 hours. The resin was then drained and washed with tetrahydrofuran/H_2O, 10% acetic acid /tetrahydrofuran, tetrahydrofuran /H_2O, tetrahydrofuran, dimethyl-formamide, dichloromethane, diethyl ether, dichloromethane, diethyl ether, and then dried *in vacuo* to give resin **5**.

A solution of PyBOP® (24 mg, 0.045 mmol) and diisopropylethylamine (16 ml, 0.09 mmol) in dimethylformamide (1 ml) was added to resin **5** (50 mg, 0.015 mmol) followed by a solution of 1-*tert*-butoxycarbonyl-1-(*o*-bromobenzyl)-diaminoethane (15 mg, 0.045 mmol) in dimethylformamide (1 ml) and the mixture was shaken for 3 days. The resin was drained and washed with dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether. A solution of 95% aqueous trifluoroacetic acid in dichloromethane (20%, 1 ml) was then added to the resin and the mixture was shaken for 2 hours. The resin was drained and washed with dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and then shaken with 10% diisopropylethylamine in dimethylformamide for 10 minutes. The resin was drained and washed with dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether. The resin (**6**) was then dried *in vacuo*.

EXAMPLE 2

Experiment to Demonstrate Residual Drug on Construct after photolysis

Scheme 2

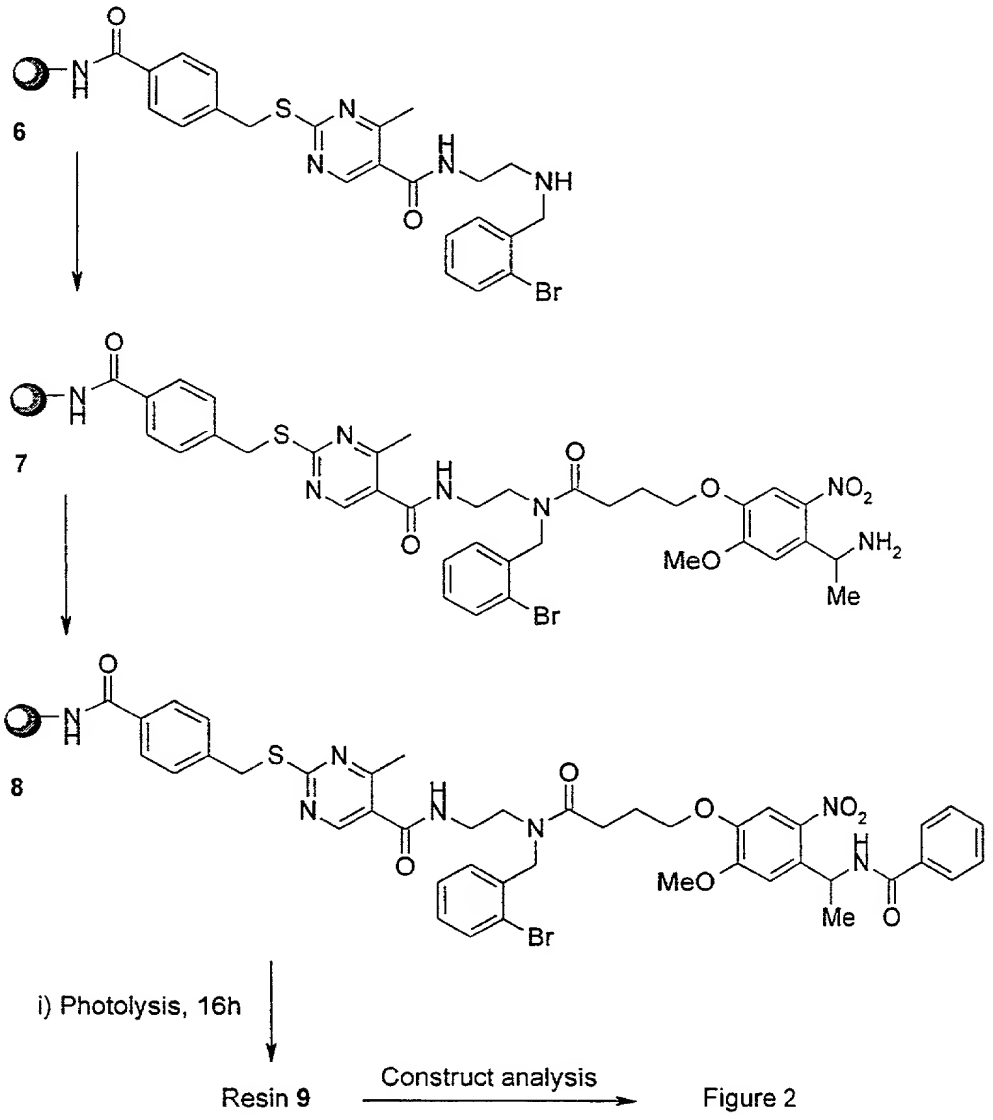


Figure 2

Experimental for Scheme 2

A solution of 4-[4-(1-(9-fluorenylmethoxycarbonylamino)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (23 mg, 0.045 mmol), diisopropylethylamine (16 ml, 0.09 mmol) and 2-(1H-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (17 mg, 0.045 mmol) in dimethylformamide (2 ml) was added to resin **6** (50 mg, 0.015 mmol) and the whole was shaken for 3 hours. The resin was drained and washed with dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether. The resin was then treated with 20% piperidine in dimethylformamide (3 ml) and the whole was shaken for 1 hour The resin (**7**) was then drained and washed with dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether and dried *in vacuo*.

A solution of benzoic acid (11 mg, 0.09 mmol), diisopropylethylamine (16 ml, 0.09 mmol) and 2-(1H-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (17 mg, 0.045 mmol) in dimethylformamide (2 ml) was added to resin **7** and the mixture was shaken for 2 hours. The resin (**8**) was then drained and washed with dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether.

Analysis of the product of the synthesis was carried out by subjecting the resin-bound construct to thiopyrimidine cleavage conditions (described below), following which the construct was analysed by mass spectrometry. The mass spectrum of the construct is shown in figure **1**.

A solution of 0.2% Hydrazine hydrate in dimethylsulphoxide (0.1 ml) was added to resin 9 (2 mg, 0.6 mmol) and the suspension subjected to photolysis for 16 hours. The photolysed resin (**9**) was then drained and washed with dimethylsulphoxide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether and dried *in vacuo*. The washed resin was subjected to pyrimidine cleavage conditions (described below) and the resulting cleavage product was analysed by mass spectrometry. The mass spectrum is shown in figure **2**.

General method of Construct Analysis of Resins via the Pyrimidine Linker.

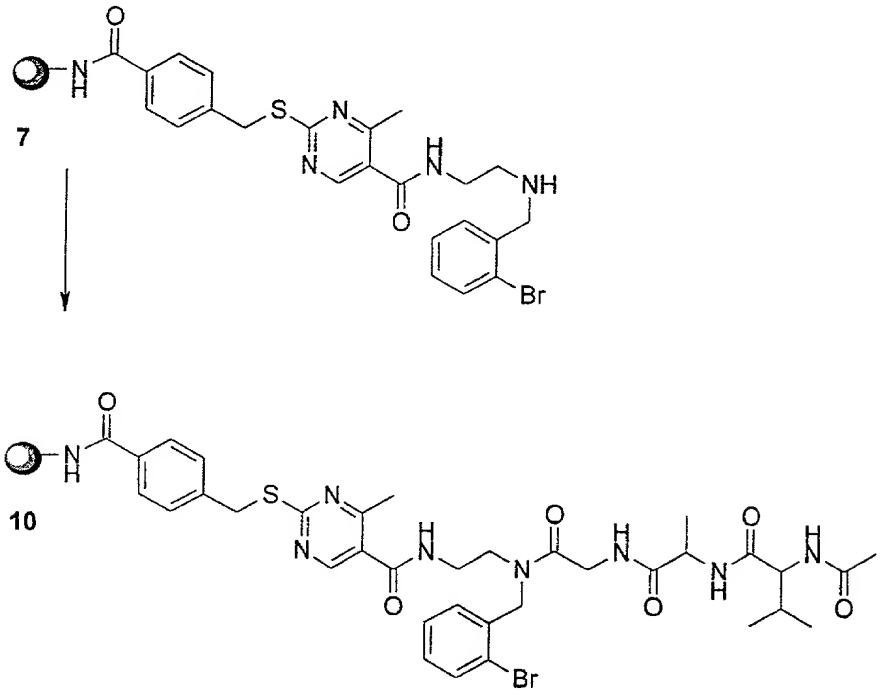
A small sample of resin (ca. 0.5 mg) is treated with 0.01M m-chloroperbenzoic acid

(0.5 ml) for 2 hours. The resin is then drained and washed with dichloromethane, diethyl ether and dichloromethane. The resin is then treated with 0.02 M N-methylpiperazine in dimethylformamide (50 ml) and the mixture is shaken for 12 hours. The solution is then removed and analysed by mass spectrometry.

EXAMPLE 3

Identification of a sensitised sequence of amino acids

Scheme 3



Experimental for Scheme 3

Resin **10** was prepared by reacting resin **7** with the desired 9-fluorenylmethoxycarbonyl protected amino acids followed by deprotection, and then repeated coupling to the next amino acid and deprotection (see general method). The terminal amino group was then acylated using acetic anhydride (see method below).

The resulting resin was subjected to pyrimidine cleavage conditions (described above) followed by mass spectroscopic analysis. The mass spectrum is shown in Figure **3**. Having identified the molecular ion at mass 716, the mass spectrum was run again at a higher voltage in order to bring about fragmentation and sequential cleavage of the three amino acids. The mass spectrum of the fragmented construct is shown in Figure **4**.

General/Typical method for coupling amino acids to the linker

A solution of the 9-fluorenylmethoxycarbonyl protected amino acid (0.14 mmol, 10 Eq), diisopropylcarbodiimide (22 ml, 0.14 mmol, 10 Eq) and N-hydroxybenzotriazole (19 mg, 0.14 mmol, 10 Eq) in dimethylformamide (1ml) was added to required resin **7** (30 mg,

ca. 0.014 mmol). The mixture was then shaken for 5 hours. The resin was then drained and washed with dichloromethane, dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether. The 9-fluorenylmethoxycarbonyl protecting groups were then removed by the treatment of the resins with 20% piperidine solution in dimethylformamide (2 ml) and shaking for 30 minutes. The resin was then drained and washed with dichloromethane, dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether. The washed resin was dried *in vacuo*.

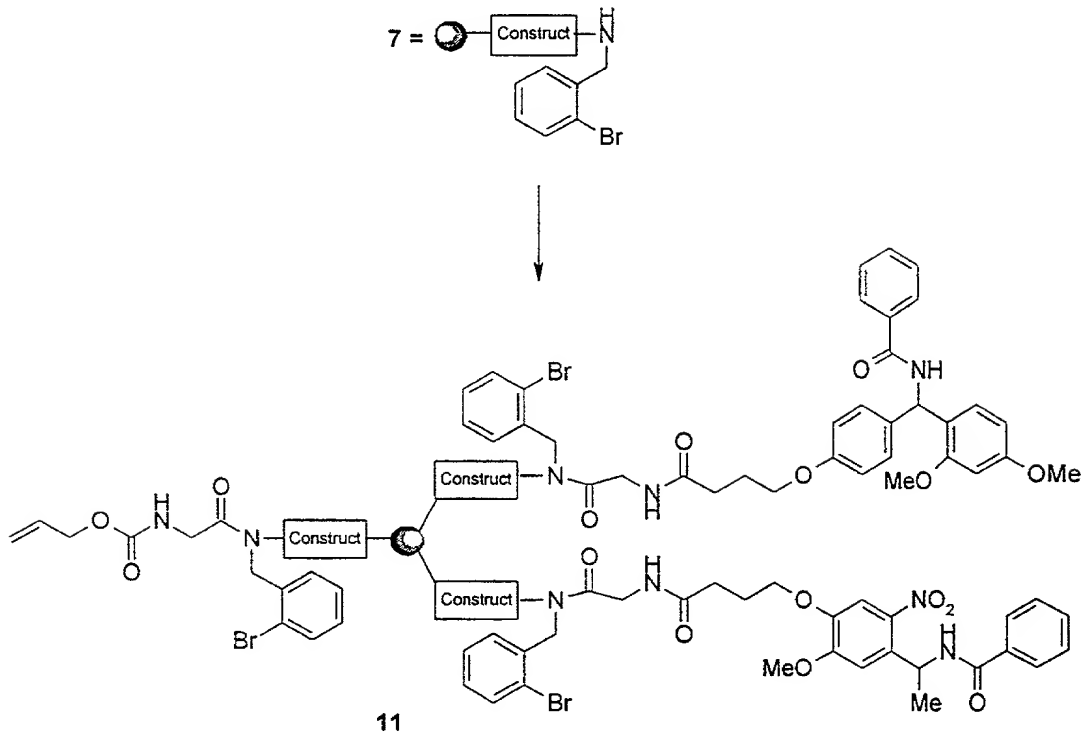
Acetylation of the dipeptides

A solution of acetic anhydride (15 ml, 0,14 mmol) in dichloromethane (1 ml) was added to the resins (30 mg, ca. 0.014 mmol) followed by 4-dimethylaminopyridine (1 mg, 0.008 mmol) and the mixture was shaken for 2 hours. The resin was then drained and washed with dichloromethane, dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether. The washed resin was dried *in vacuo*.

EXAMPLE 4

Preparation of Differential Release Resin 11

Scheme 4



11

Experimental for Scheme 4

Resin 7 (0.1 g, 0.03 mmol) was treated with a solution of 9-fluorenylmethoxycarbonyl glycine (36 mg, 0.12 mmol), N-allyloxycarbonyl glycine (5.2 mg, 0.03 mmol), PyBOP (78 mg, 0.15 mmol) and diisopropylethylamine (52 ml, 0.3 mmol) in dimethylformamide (1 ml) and the resulting mixture was shaken for 3 hours. The resin was then drained and washed with dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether and dried *in vacuo*.

The resin thus produced was treated with 20% piperidine in dimethylformamide (2 ml) and shaken for 30 minutes before draining and washing as described above. The resin was then treated with a solution of 4-[4-(1-(9-fluorenylmethoxycarbonylamino)ethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (39 mg, 0.075 mmol), p-[(R,S)-α-[1-(9H-fluoren-9-yl)-methoxyformamido]-2,4-dimethoxy-benzyl]-phenoxybutanoic acid (43 mg, 0.075 mmol), PyBOP (78 mg, 0.15 mmol) and diisopropylethylamine (52 ml, 0.3 mmol) in

dimethylformamide (1ml) and the mixture was shaken for 3 hours. The resin was then drained and washed with dimethylformamide, dichloromethane, diethyl ether, dichloromethane, and finally diethyl ether and dried *in vacuo*.

This resin was then treated with 20% piperidine in dimethylformamide (2 ml) and shaken for 30 minutes. The resin was then drained and washed as above. The resin was then treated with a solution of benzoic acid (18 mg, 0.15 mmol), 2-(1H-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (57 mg, 0.15 mmol) and diisopropylethylamine (52 ml, 0.3 mmol) in dimethylformamide (1 ml) and the whole was shaken for 2 hours. This resin was then drained and washed as above.

The product resin was subjected to pyrimidine cleavage conditions (described above) followed by mass spectroscopic analysis, and the mass spectrum thus obtained is shown in figure 4.

It will readily be apparent that numerous modifications and alterations could be made to the constructs described in the examples above without departing from the principles underlying this invention, and all such modifications and alterations are intended to be embraced by the claims appended hereto.

CLAIMS

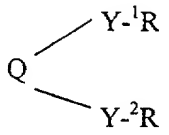
1. A chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto groups Y^1R and Y^2R; wherein R is a substrate or a coding tag and the groups Y^1 and Y^2 are connecting groups each having a first cleavage site, at least one of Y^1 and Y^2 having a second cleavage site located between the first cleavage site and group R, the first cleavage site being orthogonally and selectively cleavable with respect to the second cleavage site, and, when both groups Y^1 and Y^2 contain a second cleavage site, the second cleavage site in Y^1 being selectively and orthogonally cleavable with respect to the second cleavage site in Y^2; the second cleavage site being cleavable to release the substrate; and the first cleavage site being selectively cleavable to release a fragment Fr comprising the substrate R and at least a portion of the connecting group Y; and wherein:
(i) the chemical fragment Fr contains a sensitising group G which sensitises the chemical fragment Fr to instrumental, e.g. mass spectroscopic analysis and/or:
(ii) the fragment Fr contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

2. A chemical construct according to claim 1 wherein at least one group R is a substrate.

3. A chemical construct according to claim 2 wherein the substrate is a drug molecule.

4. A chemical construct according to claim 1 comprising a solid support Q having linked thereto groups Y^1R and Y^2R; wherein R is a substrate (such as a drug molecule) and the groups Y^1 and Y^2 are connecting groups each having first and second cleavage sites which are orthogonally and selectively cleavable, the second cleavage site in Y^1 being selectively and orthogonally cleavable with respect to the second cleavage site in Y^2; the second cleavage site being cleavable to release the substrate; and the first cleavage site being located at a position between the second cleavage site and the solid support and being selectively cleavable to release a fragment Fr comprising the substrate R and at least a portion of the connecting group Y; and wherein:
(i) the chemical fragment Fr contains a sensitising group G which sensitises the chemical fragment Fr to instrumental, e.g. mass spectroscopic analysis and/or:
(ii) the fragment Fr contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

5. A chemical construct according to claim 1 having the formula:



6. A chemical construct according to any one of the preceding claims wherein each solid support contains a coding tag or coding sequence which encodes information indicative of at least part of the synthesis history of the construct.

7. A chemical construct according to claim 6 wherein the coding tag is a coding sequence linked to the solid support.

8. A chemical construct according to claim 7 wherein the coding sequence is linked to the solid support by means of a connecting group Y^a having a cleavage site cleavable to release a fragment F^a from the solid support, the fragment F^a comprising the coding sequence and optionally at least a portion of the connecting group Y^a.

9. A chemical construct according to claim 8 wherein:
(i) the chemical fragment F^a contains a sensitising group G which sensitises the chemical fragment F^a to instrumental, e.g. mass spectroscopic analysis and/or:
(ii) the fragment F^a contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

10. A chemical construct according to claim 8 wherein the cleavage site of group Y^a is cleavable under conditions corresponding to those needed to cleave the first cleavage sites in the groups Y^1R and Y^2R.

11. A chemical construct according to claim 9 wherein the chemical fragment F^a contains a sensitising group G which sensitises the chemical fragment F^a to instrumental, e.g. mass spectroscopic analysis.

12. A chemical construct according to any one of the preceding claims wherein a proportion of the total substrate R in the construct is linked to the solid support by means of a connecting group Y^b having a cleavage site which is cleavable to release a fragment F^b

from the solid support, the fragment F^b comprising the substrate R and at least a portion of the connecting group Y^b; the connecting group Y^b not being cleavable to release substrate R under conditions effective to cleave the second cleavage sites in the groups Y^1R and Y^2R and wherein:

(i) the chemical fragment F^b contains a sensitising group G which sensitises the chemical fragment F^b to instrumental, e.g. mass spectroscopic analysis and/or:

(ii) the fragment F^b contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

13. A chemical construct according to any one of the preceding claims wherein the sensitising group G is generated by cleavage at the first cleavage site of the group Y^1 or Y^2 or, when present, Y^a or the said cleavage site of Y^b.

14. A chemical construct according to any one of the preceding claims wherein the sensitising group G is a basic amino group or a carboxylate group, preferably a basic amino group.

15. A chemical construct according to claim 14 wherein the sensitising group is a primary amino group, a secondary amino group, or a tertiary amino group.

16. A chemical construct according to claim 15 wherein the sensitising group is a tertiary amino group selected from cyclic amino groups such as piperidino, piperazino (e.g.N-methylpiperazino), pyrrolidino, or morpholino, piperidino.

17. A construct according to any one of the preceding claims wherein the fragment Fr and (where present) optionally the fragment F^a and (where present) optionally the fragment F^a contain a means for imparting a characteristic signature to the mass spectrum of the fragment.

18. A construct according to claim 17 wherein the signature is provided by incorporating into the fragment a "peak splitting" isotopic label comprising at least one atom that exists in a number of stable isotopic forms.

19. A chemical construct according to claim 18 wherein the fragments Fr and F^a, and (where present) optionally F^c are labelled differently so as to produce different characteristic

signatures.

20. A chemical construct according to claim 18 or claim 19 wherein the isotopic label comprises an atom or atoms selected from $^{1}H/H^{2}$ (D), $^{79}Br/^{81}Br$, $^{12}C/^{13}C$, $^{14}N/^{15}N$ and $^{16}O/^{18}O$.

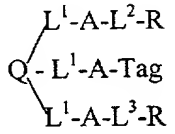
21. A chemical construct according to any one of claims 18 to 20 wherein the isotopic label(s) is/are located between the first and second cleavage sites of the groups Y^1 and Y^2.

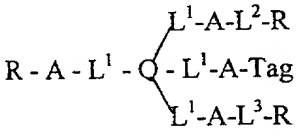
22. A chemical construct as defined in any one of the preceding claims wherein the first and second cleavage sites in the groups Y^1 are defined by first and second linker groups L^1 and L^2, first and second cleavage sites in the group Y^2 are defined by first and second linker groups L^1 and L^3, the cleavage site in the group Y^a (where present) is defined by a linker group L^a and the cleavage site in the group Y^b (where present) is defined by a linker group L^b.

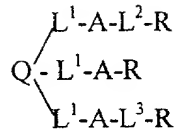
23. A chemical construct according to claim 22 wherein the linker groups L^a and L^b correspond to L^1.

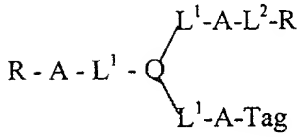
24. A chemical construct according to claim 22 or claim 23 wherein a spacer group A is interposed between each pair of first and second linker groups, or between the linker group L^a and the coding tag, or between the linker group L^b and the substrate R, the spacer group A containing an isotopic peak splitting label.

25. A chemical construct according to any one of the preceding claims having a formula selected from the group consisting of:



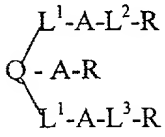


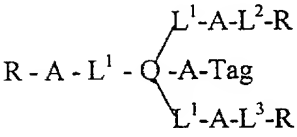


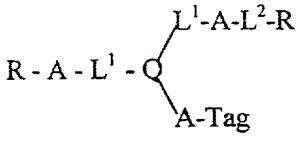


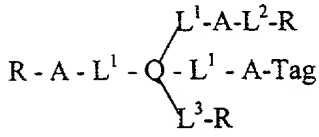
$Tag - A - L^1 - Q - L^1 - A - L^2 - R$

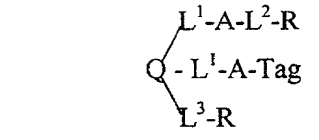
$R - A - L^1 - Q - L^1 - A - L^2 - R$

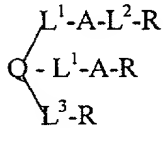




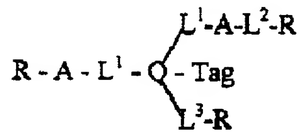








$R - L^3 - Q - L^1 - A - L^2 - R$



wherein L^1, L^2, L^3, A and R are as defined in any one of the preceding claims and "Tag" represents a coding sequence.

26. A construct as claimed in any one of claims 1 to 25 for use in a tiered release method of screening, the construct having the formula Tag - A - L^1 - Q - L^1 - A -L^2 - R wherein Tag, A, L^1, Q ,L^2 and R are as defined in any one of the preceding claims.

27. A chemical construct according to any one of the preceding claims wherein the orthogonally cleavable cleavage sites can be cleaved by a reactions selected from acid catalysed cleavage, base catalysed cleavage, oxidative cleavage, reductive cleavage, nucleophilic displacement, electrophilic displacement, and thermal, photochemical and enzymatic cleavage.

28. Intermediate chemical constructs for use preparing a chemical construct as defined in any one of the preceding claims, the intermediate constructs having the formulae $Y^{1'}$-Q-$Y^{2'}$, RY^1-Q-$Y^{2'}$and $Y^{1'}$-Q-Y^2R wherein $Y^{1'}$ and $Y^{2'}$ are reactive or protected forms of the group Y; and R, Q and Y are as defined in any one of the preceding claims.

29. Intermediate constructs of the formulae $L^{2'}$-A-L^1-Q-L^1-A^p, R-L^2-A-L^1-Q-L^1-A^p, $L^{3'}$-A-L^1-Q-L^1-A^p, R-L^3-A-L^1-Q-L^1-A^p, R-L^3-A-L^1-Q-L^1-A-$L^{2'}$ and $L^{3'}$-A-L^1-Q-L^1-A-L^2-R wherein $L^{1'}$, $L^{2'}$ and $L^{3'}$ are reactive or protected forms of the linker groups L^1, L^2 and L^3, A^p is a reactive or protected form of the spacer group A containing a peak splitting isotopic label, and Q, R, A, L^1, L^2 and L^3 are as defined in any one of the preceding claims.

30. An intermediate construct according to claim 29 wherein the group A^p has the formula NH-Alk-NX^1 wherein Alk is an alkylene group and X^1 is hydrogen or an aralkyl group.

31. An intermediate construct according to claim 29 or claim 30 wherein the solid support has bonded thereto a coding tag sequence L^1-A-Tag and/or a sequence R - A - L^1 -, or a precursor form thereof..

32. A differential release method of assaying a chemical library for biological activity, the method comprising:

(i) subjecting a construct comprising a solid support Q having linked thereto groups Y^1R and Y^2R as defined in any one of the preceding claims to cleavage conditions effective to release substrate R from the group Y^1R;

(ii) testing the substrate R released from the group Y^1R in a biological assay;

(iii) subsequently subjecting the construct to cleavage conditions effective to release substrate R from the group Y^2R; and

(iv)) testing the substrate R released from the group Y^2R in a biological assay.

33. A tiered release method of assaying a chemical library for biological activity, the method comprising:

(i) subjecting a construct as claimed in any one of claims 1 to 27 to cleavage conditions effective to release a first portion of the substrate R from the group Y^1R;

(ii) testing the first portion of substrate R released from the group Y^1R in a biological assay;

(iii) subjecting the construct to cleavage conditions effective to release a second portion of the substrate R from the group Y^1R; and

(iv) testing the second portion of substrate R released from the group Y^1R in a biological assay.

34. A method of determining the identity of a substrate R linked to a solid support Q of a construct as claimed in any one of claims 8 to 27 by mass spectrometric means; the solid support Q having a coding sequence attached thereto by means of a connecting group Y^a having a cleavage site cleavable to release a fragment F^a from the solid support, the fragment F^a comprising the coding sequence and at least a portion of the connecting group Y^a, wherein (i) the chemical fragment F^a contains a sensitising group G which sensitises the chemical fragment F^a to mass spectroscopic analysis;

the coding sequence comprising a sequence of coding groups the nature and order of which is indicative of the identity of the substrate R;

the method comprising cleaving the connecting group Y^a so as to release the fragment F^a from the solid support; subjecting the fragment Y^a to mass spectrometry under conditions effective to bring about mass spectral fragmentation of the coding group and the formation of mass spectral fragment ions corresponding to the loss of one or more coding groups from the coding sequence, and thereafter correlating mass

spectral peaks of the mass spectral fragment ions with the molecular ion of the fragment Y^a to identify the sequence of the individual coding groups.

35. A method according to claim 34 wherein the fragment F^a contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

36. A method of identifying a pharmaceutically useful substrate comprising preparing a library containing a plurality of chemical constructs as defined in any of the preceding claims, and subjecting the library to biological testing to identify biologically active substrates.

37. A method according to claim 36 that includes the further step of formulating a biologically active substrate thus identified with a pharmaceutically acceptable carrier to form a pharmaceutical composition.

ABSTRACT OF THE DISCLOSURE

CHEMICAL CONSTRUCTS AND THEIR USES

The invention provides a chemical construct for use in solid phase synthesis comprising a solid support Q having linked thereto groups Y^1R and Y^2R; wherein R is a substrate or a coding tag and the groups Y^1 and Y^2 are connecting groups each having a first cleavage site, at least one of Y^1 and Y^2 having a second cleavage site located between the first cleavage site and group R, the first cleavage site being orthogonally and selectively cleavable with respect to the second cleavage site, and, when both groups Y^1 and Y^2 contain a second cleavage site, the second cleavage site in Y^1 being selectively and orthogonally cleavable with respect to the second cleavage site in Y^2; the second cleavage site being cleavable to release the substrate; and the first cleavage site being selectively cleavable to release a fragment Fr comprising the substrate R and at least a portion of the connecting group Y; and wherein: (i) the chemical fragment Fr contains a sensitising group G which sensitises the chemical fragment Fr to instrumental e.g. mass spectroscopic analysis; and/or (ii) the fragment Fr contains a means for imparting a characteristic signature to the mass spectrum of the fragment.

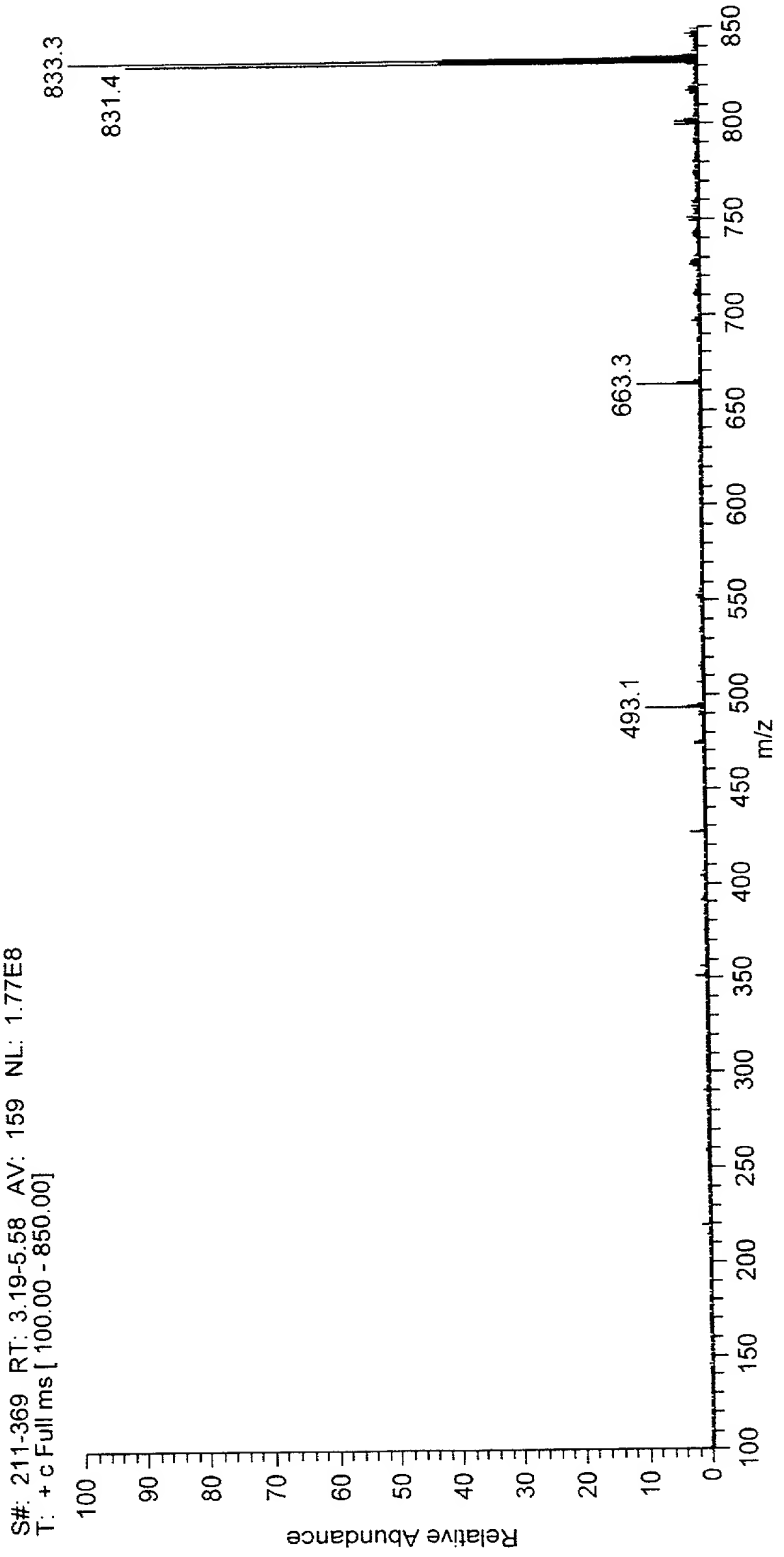


FIG. 1

L985240

FIG. 2

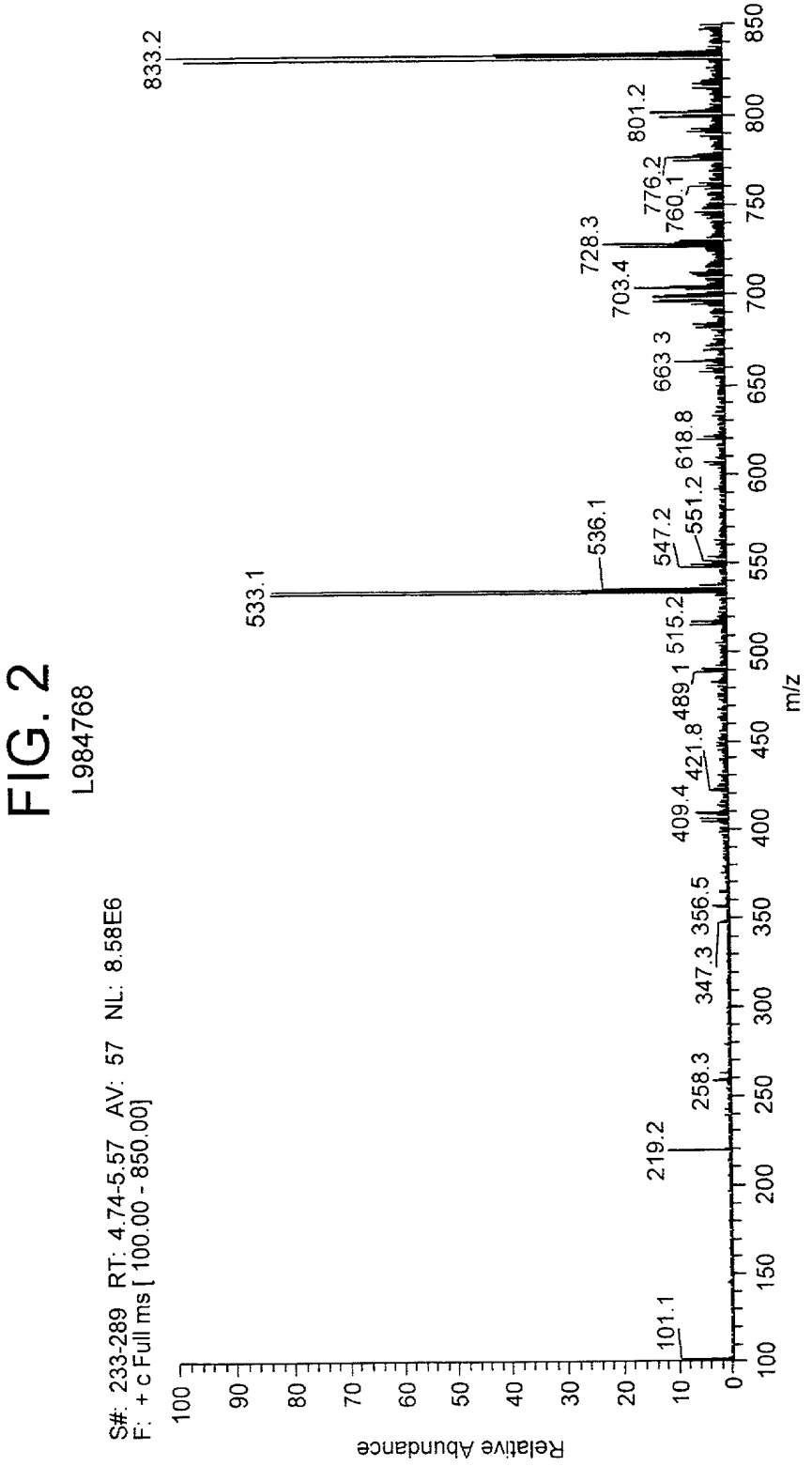

L984768
S#: 233-289 RT: 4.74-5.57 AV: 57 NL: 8.58E6
F: + c Full ms [100.00 - 850.00]
Relative Abundance
m/z
833.2
801.2
776.2
760.1
728.3
703.4
663.3
618.8
551.2
547.2
536.1
533.1
515.2
489.1
421.8
409.4
356.5
347.3
258.3
219.2
101.1

FIG. 3

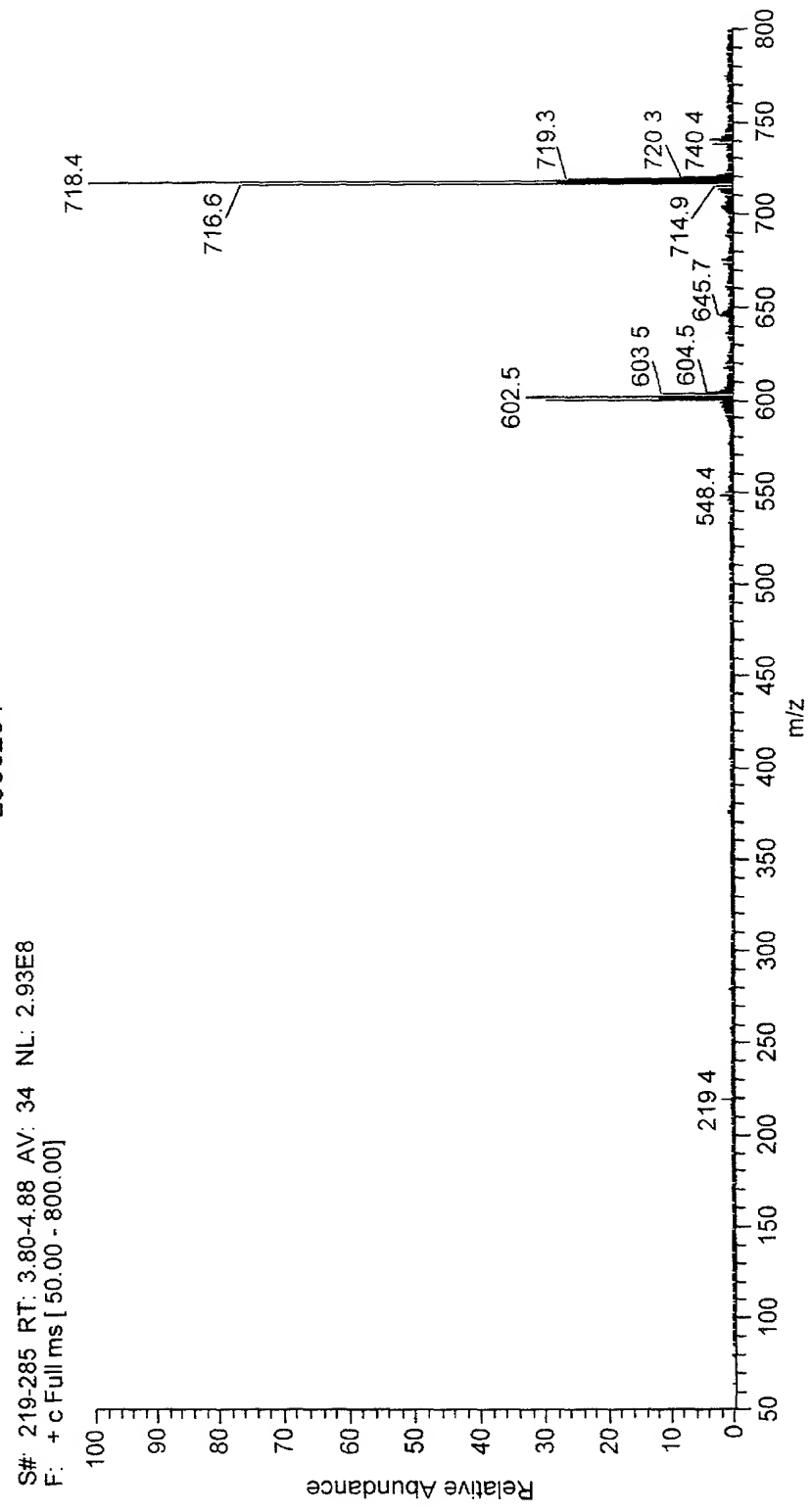

L985264
S# 219-285 RT: 3.80-4.88 AV: 34 NL: 2.93E8
F: + c Full ms [50.00 - 800.00]
Relative Abundance
m/z
718.4
716.6
719.3
720.3
740.4
714.9
645.7
603.5
604.5
602.5
548.4
219.4

FIG. 4

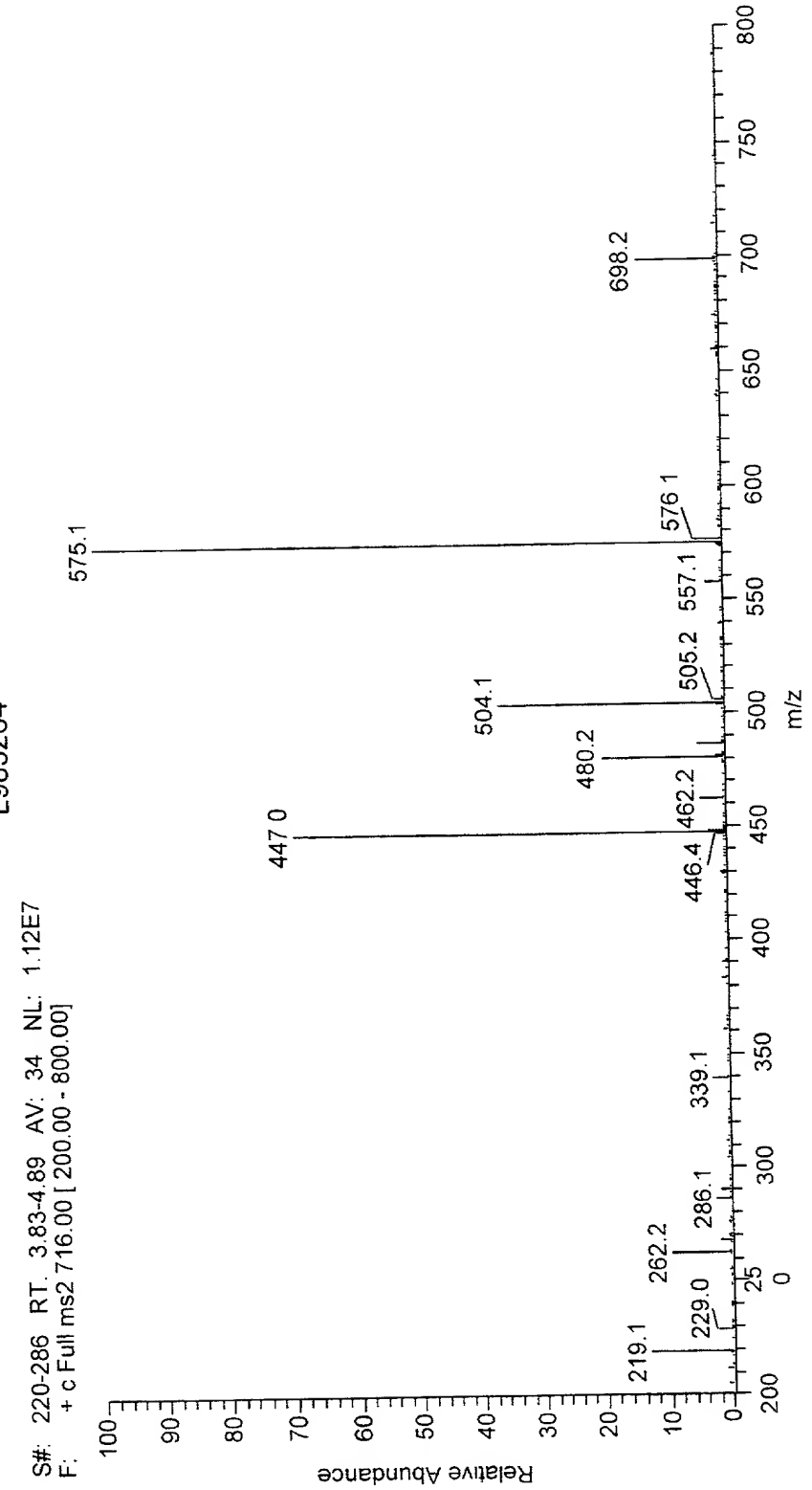
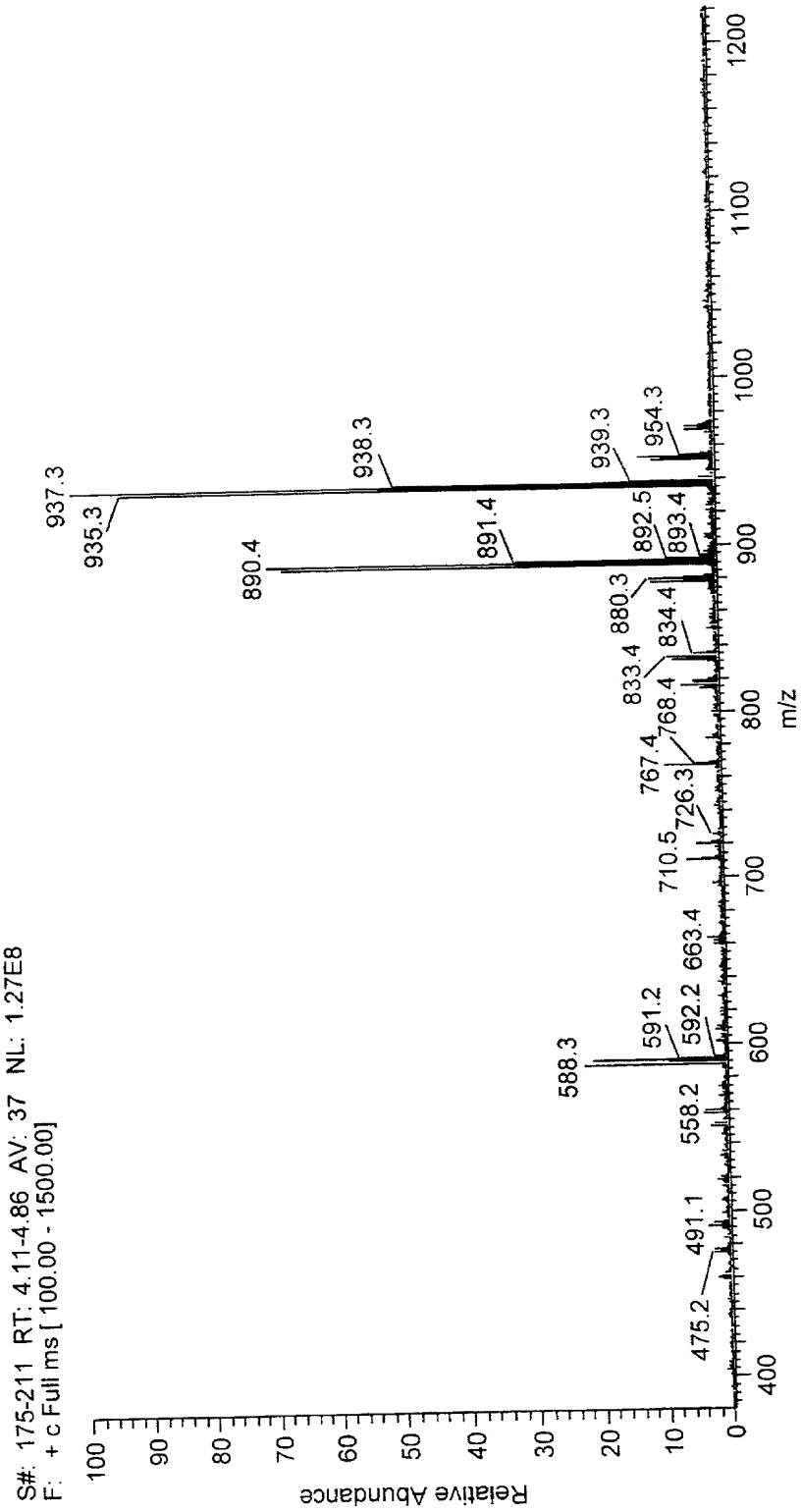

L985264
S#: 220-286 RT: 3.83-4.89 AV: 34 NL: 1.12E7
F: + c Full ms2 716.00 [200.00 - 800.00]
Relative Abundance
m/z
575.1
447.0
504.1
480.2
462.2
446.4
505.2
557.1
576.1
698.2
339.1
286.1
262.2
229.0
219.1

FIG. 5

L985510



COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY

( ) Declaration submitted with initial filing or

(X)Declaration submitted after initial filing (surcharge required 37CFR1.16(e))

ATTORNEY'S DOCKET PG3576USW
First Names Inventor: **McKeown**
Complete if known: App No.:
Filing Date Concurrently herewith
Group Art Unit:

As below named inventor. I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CHEMICAL CONSTRUCTS AND THEIR USES

the specification of which (check only one item below):

[ ]is attached hereto.
OR
[x] was filed on _____as United States application Serial No. _____ or **PCT** International

Application Number PCT/GB99/03284 filed October 5, 1999 and was amended on (MM/DD/YYYY) (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35, U.S.C. §119 (a)-(d) or §365(b) of any foreign applications(s) for patent or inventor's certificate or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

Prior Foreign Application Number (s)	Country	Foreign Filing Date (MM/DD/YYYY))	PRIORITY CLAIMED
1. 9821669.0	GB	10/05/1998	X
2.			
3.			
4.			
5.			

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date (MM/DD/YYYY)	
1.		
2.		
3.		
4.		
5.		

COMBINED DECLARATION FOR UTILITY or DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY Continued

ATTORNEY'S DOCKET NUMBER: PG3576USW

I hereby claim the benefit under 35, U.S.C. §120 of any United States application or §365(c) of any PCT international application designating the United States of America that is listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	STATUS (Check one) PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith. (List name and registration number)

David J. Levy	Reg. No. 27,655	James P. Riek	Reg. No. 39,009	Bonnie L. Deppenbrock	Reg. No. 28,209
Charles E. Dadswell	Reg. No. 35,851	Virginia C. Bennett	Reg. No. 37,092	John L. Lemanowicz	Reg. No. 37,380
Karen L. Prus	Reg. No. 39,337	Frank P.Grassler	Reg. No. 31,164	Amy H. Fix	Reg. No. 42,616
Robert H. Brink	Reg. No. 36,094	Christopher P. Rogers	Reg. No. 36,334		
Elizabeth Selby	Reg. No. 38,298	Lorie Ann Morgan	Reg. No. 38,181		

Send Correspondence to:
David J. Levy, Patent Counsel
Corporate Intellectual Property Department
GlaxoSmithKline
Five Moore Drive, PO Box 13398
Research Triangle Park, NC 27709

23347
PATENT TRADEMARK OFFICE

Direct Telephone Calls to:
Frank Grassler
919-483-2482

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2	FULL NAME OF INVENTOR	FAMILY NAME: McKEOWN	FIRST GIVEN NAME: Stephen	SECOND GIVEN NAME/INITIAL: Carl
	INVENTOR'S SIGNATURE	Stmckeown		DATE: 13/6/01
0	RESIDENCE & CITIZENSHIP	CITY: Stevenage GBX	STATE OR FOREIGN COUNTRY: GB	COUNTRY OF CITIZENSHIP: GB
1	POST OFFICE ADDRESS	POST OFFICE ADDRESS: GlaxoSmith Kline Five Moore Drive,	CITY: Research Triangle Park	STATE & ZIP CODE/COUNTRY: NC,27709 US
2	FULL NAME OF INVENTOR	FAMILY NAME: WATSON	FIRST GIVEN NAME: Stephen	SECOND GIVEN NAME/INITIAL: Paul
	INVENTOR'S SIGNATURE			DATE: 15/06/01
0	RESIDENCE & CITIZENSHIP	Stevenage GBX	STATE OR FOREIGN COUNTRY: GB	COUNTRY OF CITIZENSHIP: GB
2	POST OFFICE ADDRESS	POST OFFICE ADDRESS: GlaxoSmithKline Five Moore Drive	CITY: Research Triangle Park	STATE & ZIP CODE/COUNTRY: NC 27709 US
2	FULL NAME OF INVENTOR	FAMILY NAME:	FIRST GIVEN NAME:	SECOND GIVEN NAME/INITIAL:
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY:	STATE OR FOREIGN COUNTRY:	COUNTRY OF CITIZENSHIP:
3	POST OFFICE ADDRESS	POST OFFICE ADDRESS:	CITY:	STATE & ZIP CODE/COUNTRY: